A STUDY OF SERUM VISFATIN LEVELS, ATHEROGENIC INDEX AND BODY FAT DISTRIBUTION IN YOUNG OBESE ADULTS

Dissertation submitted to

THE TAMILNADU DR.MGR MEDICAL UNIVERSITY

In partial fulfilment of the regulations

For the award of the degree

M.D BIOCHEMISTRY BRANCH-XIII

CHENNAI MEDICAL COLLEGE HOSPITALAND RESEARCH CENTRE

IRUNGALUR, TRICHY -621105

THE TAMILNADU DR.MGR MEDICAL UNIVERSITY

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MAY-2018

CERTIFICATE

This is to certify that this dissertation entitled **"A STUDY OF SERUM VISFATIN LEVELS , ATHEROGENIC INDEX AND BODY FAT DISTRIBUTION IN YOUNG OBESE ADULTS"** is a bonafide work done by **DR.G.ANITHA** in partial fulfillment of the requirements for M.D., Branch – XIII (Biochemistry) examination of **The Tamilnadu Dr. MGR Medical University** to be held in May – 2018. The period of study was from 2015 - 2018.

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The work done by **Dr. G.ANITHA** on **"A STUDY OF SERUM VISFATIN LEVELS, ATHEROGENIC INDEX AND BODY FAT DISTRIBUTION IN YOUNG OBESE ADULTS"** is under my supervision and I assure that this candidate has abided by the rules of the Ethical Committee.

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DECLARATION

I, Dr. G.Anitha hereby solemnly declare that the dissertation title **"A STUDY OF SERUM VISFATIN LEVELS, ATHEROGENIC INDEX AND BODY FAT DISTRIBUTION IN YOUNG OBESE ADULTS"** was done by me at Chennai Medical College Hospital And Research Centre, Irungalur, Trichy, under the supervision and guidance of my Professor and Head of the Department **Dr. Kalavathy ponniraivan. M.D.,** This dissertation is submitted to Tamil Nadu Dr. M.G.R Medical University, towards partial fulfilment required for the award of M.D. Degree (Branch –XIII) in Biochemistry.

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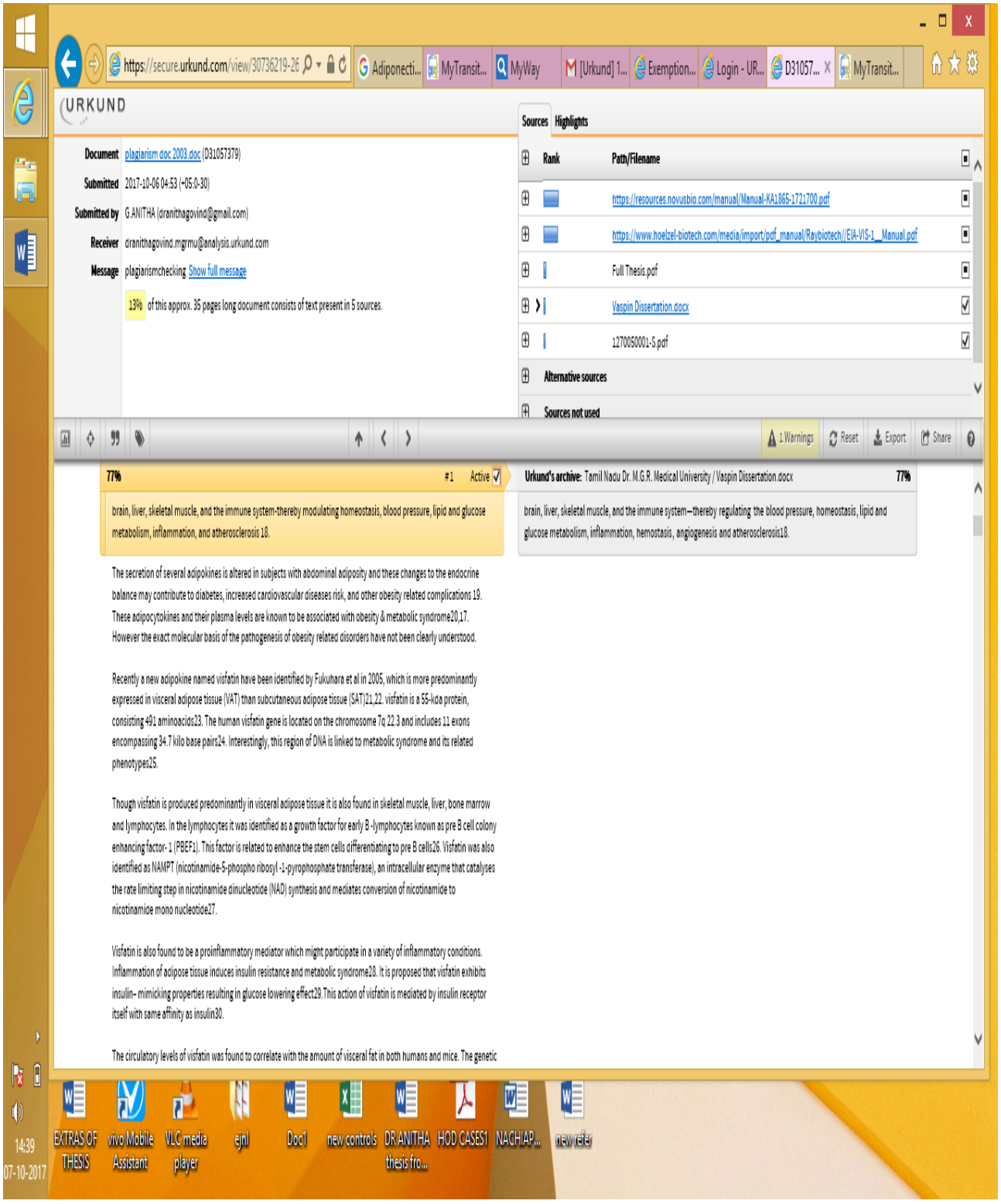
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brain, liver, skeletal muscle, and the immune system—thereby regulating the blood pressure, homeostasis, lipid and glucose metabolism, inflammation, hemostasis, angiogenesis and atherosclerosis18.
The secretion of several adipokines is altered in subjects with abdominal adiposity and these changes to the endocrine balance may contribute to diabetes, increased cardiovascular diseases risk, and other obesity related complications 19. These adipocytokines and their plasma levels are known to be associated with obesity & metabolic syndrome20,17. However the exact molecular basis of the pathogenesis of obesity related disorders have not been clearly understood.
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Though visfatin is produced predominantly in visceral adipose tissue it is also found in skeletal muscle, liver, bone marrow and lymphocytes. In the lymphocytes it was identified as a growth factor for early B-lymphocytes known as pre B cell colony enhancing factor- 1 (PBEF1). This factor is related to enhance the stem cells differentiating to pre B cells26. Visfatin was also identified as NAMPT (nicotinamide-5-phospho ribosyl -1-pyrophosphate transferase), an intracellular enzyme that catalyses the rate limiting step in nicotinamide dinucleotide (NAD) synthesis and mediates conversion of nicotinamide to nicotinamide mono nucleotide27.
Visfatin is also found to be a proinflammatory mediator which might participate in a variety of inflammatory conditions. Inflammation of adipose tissue incuces insulin resistance and metabolic syndrome28. It is proposed that visfatin exhibits insulin- mimicking properties resulting in glucose lowering effect29. This action of visfatin is mediated by insulin receptor itself with same affinity as insulin30.
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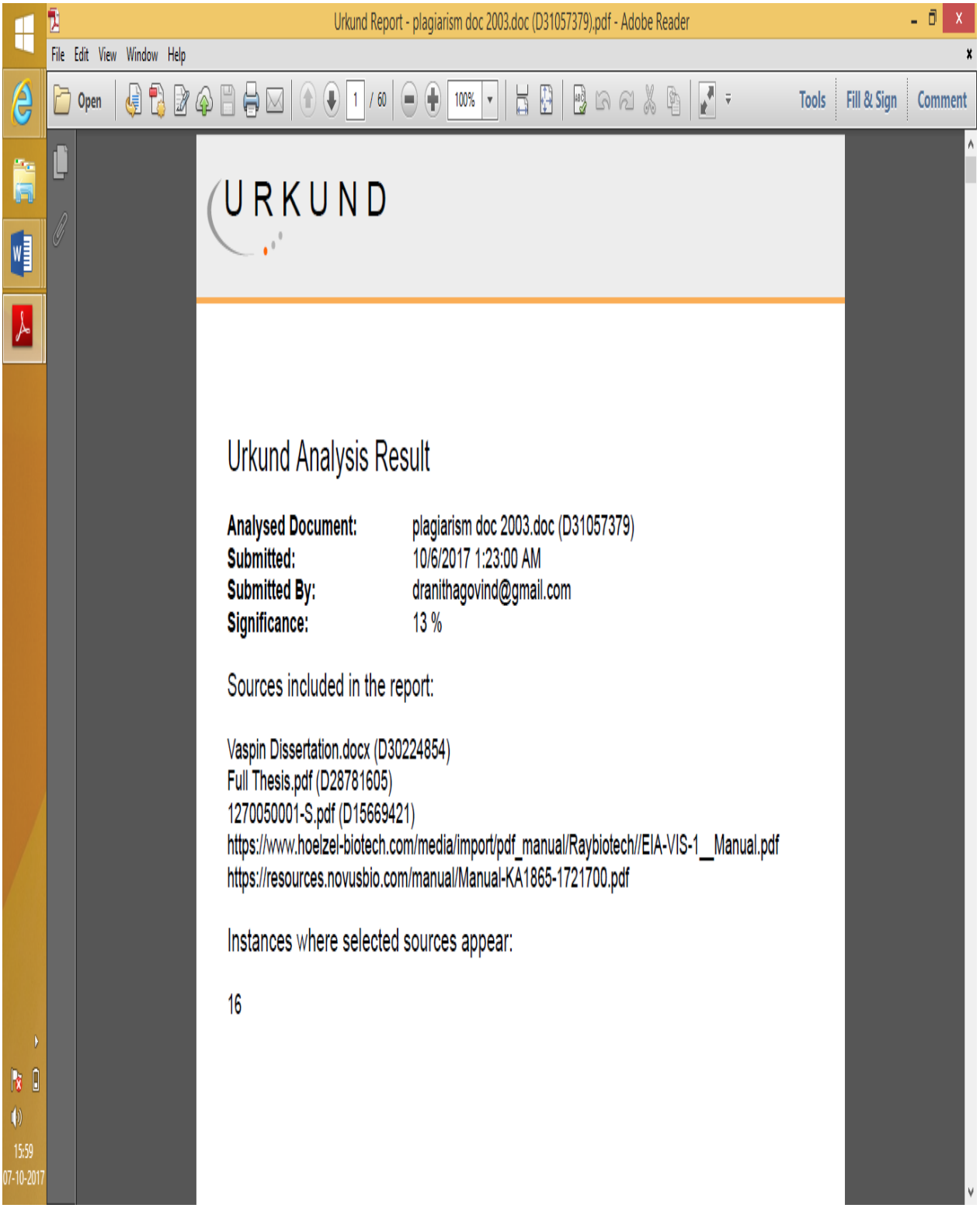
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CERTIFICATE - II

This is to certify that this dissertation work titled **"A Study of Serum Visfatin Levels, Atherogenic Index and Body Fat Distribution in Young Obese Adults"** of the candidate **Dr.G.ANITHA** with registration number **71633** for the award of **M.D DEGREE** in the branch **– XIII BIOCHEMISTRY**. I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows 13**%** percentage of plagiarism in the dissertation.

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Successful completion of my study

CONTENTS

ABBREVIATIONS

WHO	:World Health Organisation
WAT	:White Adipose Tissue
BAT	:Brown Adipose Tissue
SAT	:Subcutaneous Adipose Tissue
VAT	:Visceral Adipose Tissue
AIP	:Atherogenic Index Of Plasma
PBEF1	: Pre B Cell Colony Enhancing Factor- 1
NAMPT	:Nicotinamide-5-Phospho Ribosyl -1-Pyrophosphate Transferase
WC	:Waist Circumference
HC	:Hip Circumference
WHR	: Waist Hip Ratio
TG	: Triglycerides
LDL-C	: Low Density Lipoprotein Cholesterol
VLDL-C	: Very Low Density Lipoprotein Cholesterol
HDL-C	:High Density Lipoprotein Cholesterol
PCOS	:Polycystic Ovarian Syndrome
MDD	:Major Depressive Disorder
FFA	:Free Fatty Acid

UCP-1 : Uncoupling Protein 1

MHO :Metabolically Healthy Obese

MUHO : Metabolically Unhealthy Obese

TLR :Toll Like Receptor

PAI-1 : Plasminogen Activator Inhibitor-1

CCL-2 : C-C Chemokine Ligand-2

CT : Computerized Tomography

MRI : Magnetic Resonance Imaging

DEXA : Dual Energy X-Ray Absorptiometry

BIA : Bioelectric Impedance Assessment

BMI :Body Mass Index

INTRODUCTION

Obesity has become epidemic both in developed and developing countries worldwide[1]. The prevalence rates are continuing to increase, most rapidly in developing countries affecting all age groups[2]. According to World health statistical report 2O12 by World Health Organization (WHO), 1 in 6 adults is obese and nearly 2.8 million individuals die every year due to overweight and obesity related metabolic disorders. Among the 1.2 billion population in India, 213 million people are obese. Based on the Asian cut-offs (BMI $\geq$ 25 kg/m^2), age- adjusted prevalence of obesity in India was 45.6% in urban population and 22.5% in rural population[3].

Recent epidemiological studies have reported an increase incidence of obesity not only in adults but also in adolescents and in paediatric population[4]. The excess accumulation of fat in obesity is associated with various metabolic disorders like diabetes, dyslipidemia, hypertension and cardiovascular risks non-alcoholic fatty liver disease and also certain cancers[5].

Adipose tissue is found in mammals in two different forms: BAT (brown adipose tissue) and WAT (white adipose tissue). The main function of BAT is non shivering thermogenesis, a process of heat production through the uncoupling of oxidative phosphorylation. WAT, the white adipose tissue is the type of fat in which triacylglycerol (triglyceride) is stored and from which lipids are mobilized for systemic utilization when other tissues require energy. WAT is often divided into subcutaneous and abdominal depots, whose physiological roles in disease are distinct. Fat accumulation surrounding the abdominal viscera are called visceral fat (VAT) and fat accumulation under the skin are called subcutaneous fat (SAT)[6]. Visceral fat is strongly associated with the altered metabolic risk profile than the fat accumulation

under the skin subcutaneous fat. Thus body fat distribution plays an important role in the pathogenesis of the metabolic disorders associated with obesity[7].

One of the major metabolic dearrangement of obesity is altered lipid metabolism leading to higher levels of plasma cholesterol and low density lipoproteins called Dyslipidaemia. Dyslipidaemia consequently leads to deposition of fat in the subendothelium of blood vessels which causes atherosclerosis[8]. Atherosclerosis in the major blood vessels causes stroke, myocardial infarction, renal failure and death. Atherosclerosis in the microvessels leads to infarction, gangrene as in the case of diabetes and cardiovascular diseases[9].Among the various markers of atherosclerosis, **Atherogenic Index of plasma** has proved to be the significant predictor of atherosclerosis and dyslipidemia. The Atherogenic Index of plasma is calculated using the formula log (TG/HDL)[10].

Adipose tissue, in addition to adipocytes, also contains pre-adipocytes, endothelial cells, fibroblasts and various leucocytes, including macrophages. These macrophages are sources of soluble mediators in the adipose tissue. These macrophages are bone-marrow derived and the number of these cells present in WAT directly correlates with obesity[11]. Adipose tissue is no longer considered as an inert organ that functions only for energy storage.[12] They secrete various soluble mediators called adipocytokines, which exert their biological function in an autocrine, paracrine or systemic manner[13]. This group of mediators is growing rapidly and is currently thought to represent a major link between the adipose tissue and the immune system[14]. These adipocytokines include adiponectin, leptin, vaspin, resistin, omentin, TNF (tumour necrosis factor)-α, IL (interleukin)-6,CCL-2 [C-C chemokine ligand-2; also known as MCP-1 (monocyte chemoattractant protein-1)], PAI-1 (plasminogen

activator inhibitor-1), angiotensinogen, RPB-4 (retinol-binding protein-4), SAA (serum amyloid A) and others [15,16,17].

Adipocytokines, the chemical mediators secreted by the adipose tissue have signalling pathways to organs such as brain, liver, skeletal muscle, and the immune system-thereby modulating homeostasis, blood pressure, lipid and glucose metabolism, inflammation, and atherosclerosis [18]. The secretion of several adipokines is altered in subjects with abdominal adiposity and these changes to the endocrine balance may contribute to diabetes, increased cardiovascular diseases risk, and other obesity related complications[19]. These adipocytokines and their plasma levels are known to be associated with obesity & metabolic syndrome[20,17]. However the exact molecular basis of the pathogenesis of obesity related disorders have not been clearly understood.

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The circulatory levels of visfatin was found to correlate with the amount of visceral fat in both humans and mice. The genetic expression of visfatin and thereby the plasma levels of visfatin increase in parallel with obesity and more precisely with increase in visceral fat distribution[31].

The association between visfatin levels and the body mass index has not been clearly established in the previous studies[32,33]. Further there is lack of consensus regarding the plasma visfatin levels and their relationship with atherogenic index and anthropometric measurements.This present study aims to investigate the association between serum visfatin levels, atherogenic index and body fat distribution in young obese adults.

REVIEW OF LITERATURE

OBESITY

Obesity results from an imbalance between food intake and energy expenditure, which leads to excessive accumulation of adipose tissue and lipogenesis[34]. Obesity is a complex metabolic disorder which links biologic, psychosocial, genetic, epigenetic, socioeconomic status and behavioral factors [35]. Accumulating evidences indicates that obesity is closely related to many co-morbid Conditions like Insulin resistance and Type2 Diabetes, Dyslipidaemia, Non-alcoholic fatty liver disease, Hypertension, Cardiovascular diseases and Deep vein thrombosis[36].

Obesity can be seen as the first wave of a defined cluster of non-communicable diseases called "New World Syndrome," creating an enormous socioeconomic and public health burden in poorer countries[37].

INCIDENCE AND PREVALENCE OF OBESITY

The Prevalence of Obesity has increased dramatically over the last 20 years and continue to do so, primarily as a result of changes in calorie intake and exercise patterns. Recent decades have seen an unprecedented rise in obesity, and the health impact thereof is clearly evident[38].

WORLDWIDE PREVALENCE:

The World Health Organization (WHO) have described Obesity as the most concerning and blatantly visible – yet most neglected public health problem around the globe. Obesity has become the major global health issue with significant morbidity and mortality. Obesity and overweight are the fifth

leading cause of global deaths and about 2.8 million adults die each year as a result of being obese and due to its metabolic complications[39].

According to WHO statistics 2016, worldwide, more than 2.3 billion adults are overweight (Body Mass Index [BMI] 25-29.9 kg/m^2), and of these, over 700 million are obese (BMI $\geq$ 30 kg/m^2)[40].

In the United States OF America, data from NHANES, National Health and Nutrition Examination Survey 2014, about 36.5% adults are obese and 17% of children and adolescents of age groups 2 -19 years are obese[41]. This Obesity pandemic which first started in developed countries have encroached the developing countries as well and the reasons attributed may be urbanization, increased food intake, and reduced physical activity[42].

Asians, particularly have a higher risk of developing obesity and obesity related diseases, such as Insulin resistance, the Metabolic syndrome, Type-2 Diabetes Mellitus (T2DM) and Coronary Heart Disease (CHD). The determinants that makes Asians more susceptible for obesity are their specific genotype and their body phenotype such as high body fat, high truncal obesity, subcutaneous and intra-abdominal fat, low muscle mass and insulin resistance[43.] This leads to the need of setting lower BMI cut-offs for Asians by the WHO and the BMI cut –off for obesity in adults was reduced from

$\geq$ 30 to $\geq$ 25 for asians[44].

INDIAN SCENARIO

In India, the nation that harbours the two extremes of malnutrition – Obesity and Malnutrition, shows an increasing trend of obesity problem over the recent years. Epidemiological studies have shown that Indians, being Asians are also prone to have central obesity and glucose intolerance, that contribute for the increasing trend of co-morbidities like insulin resistance, Type 2 Diabetes mellitus and coronary artery diseases in India[45].

India, the second most populous country in the world is currently experiencing rapid epidemiological and nutritional transition. Studies from different parts of India have provided evidence for the rising prevalence of Obesity[46].

According to National Family Health Survey (NFHS) in India, about 40 Million adults accounting for 18% of the country‘s population, are obese[47.] Around 20 percent of children aged 2-12 years and 3.6 percent in adolescent age-group of 13–18 years are obese. India is currently in the fourth phase of nutritional transition where there is a shift of nutritional intake from basic ancestral diet to high-fat diet that is related non-communicable diseases. These shifts are largely associated with behavioral changes, lifestyle changes, dietary profile, and decreased indulgence in physical activity[48].These transitions are becoming more rapid in younger individuals. This increasing trend of childhood Overweight and Obesity may further increase the enormous burden of Type 2 Diabetes and Cardiovascular diseases in India and would furthermore impact the economy of the nation and its growth[49].

In India, the prevalence of obesity is increased in urban population (13-50%) as compared to rural population (8-38.2%) . Studies have revealed that in a decade, the prevalence of Obesity had increased by 1.7-fold in the urban set up[50].

CHILDHOOD OBESITY

Obesity affects different age groups of people extending right from the infancy, childhood, adolescent period and adults[51]**.** Obesity presents as a substantial health problem, even for very young children. Obesity and excessive weight gain in the first year of life are associated with elevated blood pressure,[52] wheezing[53] and other adverse health conditions in childhood . Infants who gain weight more quickly in the first year of life are more likely to be obese later in childhood[54].

According to the WHO, the number of Overweight & Obese young children (aged 0 to 5 years) increased globally from 32 million in 1990 to 42 million in 2014. The rate of increase in the overweight or obese children had been more than 30% higher in developing countries, compared to that of developed countries.

India has the second highest obese children in the world, next to china and about 14.4 million children are obese in India[55].

Though genetic susceptibility can be one of the cause of childhood obesity, various studies have revealed that the unhealthy dietary habits of consuming junk foods, sugary beverages, fast foods which are less nutritious and have high calories, have become the major contributing factor for the development of childhood obesity [56]. The obese children who were over 5

years of age, incurred a greater risk of persisting obesity, with the majority remaining obese in adult life [57].

SEX PREPONDERANCE IN OBESITY

The Prevalence of Obesity is higher in women than in men in most countries around the world. According to WHO global statistics 2015, 15% of women are obese when compared with obese men who accounted for only 11% [58.]

In India, based on the reports of National Family Health Survey (NFHS), the percentage of women aged 15-49 years who are overweight or obese increased from 11% to 15% [59]. Indian studies have shown that women with increased age, house wives, women belonging to upper socio-economic strata and native Punjabi origin were found to be at greater risk for developing obesity[60].

Another study have attributed the reasons for the sex preponderance in women to the fact that women have more fat tissue and men have greater ability to deposit more lean tissue than fat tissue. The lean tissue that comprises of the bone mass and muscle mass is metabolically active and increases the basal metabolic rate in men, thereby compensating for the discrepancy between energy intake and output. Women are naturally fatter, with less lean tissue and more fat tissue than men, gain more weight in adapting to the excessive intake of calories[61].

OBESITY – ETIOLOGY

Though the exact cause of obesity is unknown, there appears to be a complex interplay among biologic, behavioral and psychosocial factors, which include socioeconomic status genetic makeup and cultural influences. Obesity has been linked to microorganisms, epigenetics, increasing maternal age, lack of sleep, endocrine disruptors, microorganisms, pharmaceutical agents, and intrauterine effects[62].

Environmental and behavioral factors have a greater influence on the development of obesity. Consumption of excess calories from fatty foods and restricted physical activity over the long run will lead to weight gain. This tremendous increase in obesity over the past 30 years have been fueled by the combined effect of social, economic, environmental and behavioral factors, acting on the background of genetic susceptibility.

Food Choices and Influence on Weight

Foods that have increased fat, salt, simple sugar and decreased fiber have high calories are more readily available, and are relatively cheaper than healthier food alternatives. Consumption of these ultra-processed foods, fast foods and junk foods has led to increase of approximately 205-calories in an average daily caloric intake of an individual[63].

Socioeconomic Factors and Obesity

Population segregation in metropolitan areas and people living in high socioeconomic strata have increased risk of developing obesity. Reduction or lack of physical activity also contributes to weight gain people in high socio-economic status and those residing in cities, avoid walking and choose car or flight for transportation.

Genetics and Obesity

BMI and body fat distribution is strongly correlated with genetic factors . It appears that genetics determine who will become obese, and the environment determines the extent of obesity in an individual[62].

Gut Microbiome

The body's microbiome such as viruses, bacteria, archaea, and eukaryotic microbes residing in the body have the potential to influence human physiology in a number of ways, including various metabolic pathways and signaling pathways related to appetite control and obesity[62].

Chrono disruption

Chrono disruption is disturbance in the circadian organization of endocrine and metabolic functions induced by lack of sleep, shift work, or shift in the normal time of eating to night hours. It is associated with the development of obesity, prediabetes, diabetes, and lipid disorders[64].

Relationship between Hormones and Weight

Many neural and hormonal signals between the gut and Central Nervous System (CNS) is regulated by the intake of food. Hormones, such as glucagon-like peptide (GLP), leptin, peptide tyrosine-tyrosine (PYY), oxyntomodulin (OXM), and cholecystokinin (CCK), signal to important areas in the central nervous system are involved in appetite control. Concentrations of these hormones increase in the blood after intake of food and these concentrations are proportional to the intake of calories and composition of the meal[65].

- **Adiponectin**

  Low levels of adiponectin is associated with weight gain and obesity. Adiponectin stimulates fatty acid oxidation and increases insulin sensitivity in the tissues.It induces glucose and lipid metabolism in insulin-sensitive tissues. Insulin resistance and type 2 diabetes mellitus are associated with decreased plasma concentrations of adiponectin[65].

- **Ghrelin**

  Obesity is associated with increased ghrelin concentrations. Ghrelin, a potent orexigenic hormone stimulates increased food intake. The levels of ghrelin are elevated 1 to 2 hours before meals and are decreased soon after. The function of this hormone is to increase the desire of food intake, increase the fat metabolism and to reduce the resting energy expenditure[62].

Hypothyroidism

The interrelation between hypothyroidism and obesity is a complex one with hypothyroidism affecting weight and obesity affecting thyroid function. Thyroid hormones are closely integrated to body composition as they regulate basal metabolism and thermogenesis. Thyroid hormones affect glucose and lipid metabolism and thereby the food intake[66].

Cushing's syndrome

Patients who develop Cushing's syndrome due to excess gluco-corticoids concentrations in adrenal tumors or long term steroids therapy are prone to develop sudden weight gain and central obesity[62].

Polycystic Ovary Syndrome(PCOS)

Females with PCOS are characterized by irregular menstrual periods, ovarian cysts , infertility, excess hair growth ,acne prone skin, ovarian cysts, patches of dark thickened skin and obesity.

MEDICATIONS

Medications such as antidepressants, beta-blockers, atypical antipsychotics, and antiepileptic drugs have been associated with weight gain and obesity[62].

- **Anti -depressants and anti-psychotics**

  Anti-depressants used for the treatment of major depressive disorder have increased risk of overweight and obesity.Antipschotics which are also used in Major Depressive Disorder (MDD) are associated with diabetes, lipid disorders and obesity. Women who are on anti-depressants were more likely to develop obesity than me

- **Antiepileptic Drugs**

  Antiepileptic Drugs such as Valproic acid, gabapentin and carbamazepine are known to be associated with weight gain and obesity. Significant weight gain of 5- 50 kg have reported with long term intake of Valproic acid.

- **Corticosteroids**

  Though Steroid therapy are important anti-inflammatory agents; they are known to be associated with weight gain and obesity[62].

- **Beta-blockers**

  Beta-blockers are drugs used in the management of hypertension and heart failure and for prophylaxis of myocardial infarction and migraine , have also been associated with a mean weight gain of 1.2 kg[62].

CONSEQUENCES OF OBESITY

Obese individuals have an increased risk of morbidity from Dyslipidemia, Hypertension T2D, Coronary Heart Disease, Stroke, Respiratory problems, Sleep Apnea, Osteoarthritis, Gallbladder Disease and some cancers[69].

Diabetes mellitus, insulin resistance, and the metabolic syndrome.

Overweight and obesity are associated with 65% of cases of type2 DM. diabetes. The risk of type 2 diabetes mellitus increases with the degree and duration of obesity and more with visceral distribution of body fat increased insulin secretion and insulin resistance result from obesity. Insulin resistance is the predominant feature in metabolic syndrome. Increased visceral fat accumulation is the central feature of this syndrome. Alternatively, weight loss or moderating weight gain over years have proved to reduce the risk of developing Type2 DM [69].

Hypertension

Overweight and obese individuals often have increased blood pressure. Hypertension is associated with increased preload and stroke work. Hypertension and obesity together have a higher risk for cardiac failure.

Obesity also affects the kidney and can cause hypertensive glomerulopathy and leads to end stage renal disease.

Heart disease

Patients with BMI greater than 30kg/m^2 often have increased risk of heart disease. There have been a positive correlation between BMI and dyslipidemias. Dyslipidemia, described as elevated plasma triglycerides, total cholesterol, LDL-C,VLDL-C and low HDL-C have been associated with increased risk of atherosclerosis and coronary artery disease[69].

Nonalcoholic fatty liver disease (NAFLD) and Nonalcoholic steatohepatitis

Obesity is often associated with fatty liver, steatosis and steatohepatitis. Weight gain are also linked to other liver disorders like hepatomegaly, elevated liver enzymes and fibrosis[69] .

Gallbladder disease.

Cholelithiasis is the primary pathology in the hepatobiliary tract which is associated with obesity. Incidence of cholelithiasis is increased gradually with increased BMI of more than 30 kg/m^2.

Obstructive sleep apnea

Obesity is associated with Sleep apnea and it is more common in men than women. Snoring is the classical feature of sleep apnea. Increased neck circumference and fat deposition in the para pharyngeal areas leads to obstructive sleep apnea [62].

Skin disorders

The common skin changes associated with obesity are Acanthosis nigrans characterized by pigmentation in skin folds of neck, knuckles, and extensor surfaces[69]

Bone disorders

Obesity is frequently associated with chronic debilitating conditions such as osteoarthritis and rheumatoid arthritis. This may be due to increased strain to the weight bearing regions in obesity[69].

Non-allergic Rhinitis

Both children and adults who are overweight or obese have an increased preponderance of developing nonallergic rhinitis[67].

Major Depressive Disorder

Obesity is also a risk factor for psychiatric illnesses such as Major Depressive Disorder (MDD) and are more common in women with BMI of 30 or higher[6].

Cancer

Obese individuals have an increased risk of developing various cancers. Higher BMI are positively correlated with an increased risk of development of uterine, gallbladder, kidney, cervical, and thyroid cancers, along with leukemia, and positive associations were seen with liver, colon, ovarian, and postmenopausal breast cancers[68].

PATHOGENESIS OF OBESITY

Adipose tissue excess and adipose tissue dysfunction play an important role in the development of obesity and obesity related metabolic complications. Positive energy balance causes adipose tissue to store excess energy as lipid droplets of triglycerides in the adipocytes. Obesity is characterized by enlargement of the size of adipocytes (hypertrophy).

The number of adipocytes is determined in childhood and remains constant during adulthood in both lean and obese. Hence increase in fat mass in adulthood is attributed to the hypertrophy of adipocytes rather than hyperplasia of adipocytes (increase in number of adipocytes).

In the normal state, when body needs energy during exercise between meals, triglycerol stored in the adipocytes are mobilized by lipolysis to release free fatty acids (FFA) into circulation. Thus FFAs are transported to other tissue and utilized for energy[70].

In obesity, FFAs enter the liver via portal circulation and increased FFA in the liver induce increased lipid synthesis, gluconeogenesis and insulin resistance in the liver. FFA, which are products of lipolysis, play a critical role in the development of insulin resistance and other obesity related metabolic complications.

FFAs act as ligands for TLR receptor (toll like receptor) complex and induce production of various cytokines responsible for inflammation of the adipose tissue which is responsible for many obesity related metabolic complications[70].

ADIPOSE TISSUE

Adipose tissue consist of various types of cells that includes lipid filled adipocytes, pericytes, preadipocytes, endothelial cells, fibroblasts, mast cells and immune cells (macrophages and T cells).

There are two types of adipose tissue in mammals, the white adipose tissue and brown adipose tissue. There are significant differences between the two types of adipose tissue in their distribution, morphology and function[71].

WHITE ADIPOSE TISSUE (WAT)

White adipose tissue is distributed in different compartments over the entire body, and is the main energy reservoir and secretes various adipokines that regulate body metabolism and insulin sensitivity.

The adipocyte of white adipose tissue is spherical, sized between 25 to 200μm and it has a peripheral flat nucleus with a thin cytoplasm which has a single large lipid drop, that occupies 90% of the cell . It has few mitochondria and are poorly vascularized[71].

BROWN ADIPOSE TISSUE (BAT)

Brown adipose tissue, though initially thought to be present only in neonates, it was found to be present in adults also in paravertebral, supraclavicular cervical, paravertebral, para-aortic, mediastinal and adrenal regions. The main function of BAT is energy expenditure through non-shivering thermogenesis through the mitochondrial uncoupling protein 1(UCP-1). The adipocyte of brown adipose tissue is polygonal with an oval, centred nucleus on a large cytoplasm with multiple small lipid droplets. It has large number of mitochondria and highly vascularized.

Besides thermogenesis, recent studies have shown that BAT may be associated with the reduction of triglyceride levels and plasma glucose levels[71].

BODY FAT DISTRIBUTION

Body fat distribution is a strong metabolic risk factor that is associated with obesity related co – morbidities and mortality. There are two types of distribution of white adipose tissue in humans namely,

- **Central or android obesity:**

  In this type, fat accumulates in the upper abdomen, surrounding the viscera. This type is also called as visceral/abdominal obesity.

- **Peripheral or gynoid obesity:**

  In this type, fat accumulates in the gluteo-femoral region, in the subcutaneous region[71].

MHO (Metabolically Healthy Obese) AND MUHO (Metabolically Unhealthy Obese)

Based on the body fat distribution and their metabolic implications, obese individuals are divided into two subgroups as Metabolically healthy obese (MHO) and Metabolically unhealthy obese
(MUHO).

Metabolically healthy obese individuals (MHO) have less visceral fat and more subcutaneous or peripheral fat and they are relatively gives protection from cardiovascular complications and insulin resistance.

Metabolically unhealthy obese individuals (MUHO), are characterized by less amount of subcutaneous fat , high visceral fat depots and inflammation of the visceral fat that contributes to the development of insulin resistance and cardiovascular risks.

The inter-individual differences in body fat distribution are complex and are determined by various factors like genetic, epigenetic mechanisms, sex hormones and intake of glucocorticoids[72].

MEASUREMENTS OF OBESITY

There are several methods that body fat can be measured. The most common methods to measure the levels of adiposity are BMI, Waist circumference, Waist-hip ratio, Bioelectric impedance (BIA), Densitometry (under water weighing), Air-displacement plethysmography, Dilution method (Hydrometry), Dual energy x-ray absorptiometry, Computerized tomography and magnetic resonance imaging. These methods have their own strengths and limitations[73].

BODY MASS INDEX (BMI)

BMI provides the standard cut-off for overweight and obese status. WHO classification of obesity is primarily based on BMI. BMI is calculated by measuring height and weight by using accurate standard methods. Many studies have shown that high BMI strongly co-relates with high cardiovascular risks and early death.

Strengths: It is easy to measure and inexpensive.

Limitations: BMI is an indirect method of estimating body fat. It does not distinguish body fat and lean body mass.At the same BMI cut-off, women may have more body fat than men as men have more lean body mass (Bones, Muscles) than women.

WAIST CIRCUMFERENCE:

Waist circumference is simple and common method for measuring abdominal obesity. The fat in the central body is an important factor implying disease, independent of BMI. Waist circumference is measured between lowest rib and the superior part of pelvic bone, at the level of umbilicus.

Strengths: It is inexpensive, easy to measure and strongly co-relates with body fat.

Limitations: Difficult to measure and less accurate in individuals with BMI 35 or higher[73].

WAIST-HIP RATIO:

Waist-hip ratio is used for measuring abdominal obesity and has good correlation with obesity complications. Waist-hip ratio is increased by increased abdominal fat around the hips or in central obesity.

Strengths: WHR is inexpensive and has good correlation with body fat when measured by standard methods.

Limitations: Measurements errors are common as it involves two measurements. Difficult to measure in individuals with BMI 35 or higher.

SKINFOLD THICKNESS:

A special calliper is used in this method to measure the thickness of a pinch of skin and the fat beneath it in specific areas such as thigh, trunk, back of upper arm. The body fat percentage is calculated using equations, based on these measurements.

Limitations: This method is not reproducible and not very accurate[73].

BIOELECTRIC IMPEDANCE ASSESSMENT (BIA)

BIA equipment passes an imperceptible, minimal safe electric current through the body and the resistance is measured. The current counters more resistance when it passes through body fat than it when it passes through lean body mass or body fluids. The body fat percentage and fat-free mass is calculated using equations.

Strengths: BIA is safe, portable, convenient method.

Limitations: This method may not be accurate when the ratio of body fat to body fluids changes during dehydration, illness or weight loss[73].

DENSITOMETRY (UNDER WATER WEIGHING):

Individuals are weighed under water (submerged in a tank) and weighed in air. Both the measurements are compared and body fat percentage is calculated using specific formulas. This method works in the principle that high body fat in a person would have lower body density than a person with low body fat. This method is not for routine use and only for research purpose

Strengths: It is very accurate.

Limitations: Time consuming and not suitable for children and older adults as it requires individuals to be submerged in water.

AIR-DISPLACEMENT PLETHYSMOGRAPHY

This method uses the same principle as underwater weighing but weighing is done in the air instead of water. Individuals sit in a small chamber air pressure differences between the occupied chamber and empty chamber is measured and the total body volume is estimated.

Strengths: It is comfortable for children, older individuals, pregnant women and for those with BMI 40 or higher.it is accurate and safe[73].

Limitations: This method is expensive.

DILUTION METHOD (HYDROMETRY)

This method is based on the ratio of body water and fat free mass. Individuals are made to drink isotope-labeled water and their body fluid samples were analysed for isotope levels which are used to calculate total body water, fat free mass and total body fat mass.

Strengths: Low cost, accurate, and used in individuals with a BMI of 40 or higher.

Limitations: The ratio of body water to fat-free mass may change during illness, dehydration, or weight loss and affect the accuracy of the method[73].

DUAL ENERGY X-RAY ABSORPTIOMETRY :(DEXA)

X-ray beams penetrate different body tissues at different rates and DEXA uses two low-level X-ray beams to measure fat-free mass, fat mass, and bone density.

Strengths: DEXA is accurate.

Limitations: Equipment is expensive and not portable, and this method does not differentiate visceral and subcutaneous fat.It cannot be used in pregnant women

COMPUTERIZED TOMOGRAPHY AND MAGNETIC RESONANCE IMAGING:

These imaging modalities are the most accurate methods for measuring whole-body fat mass, lean muscle mass and bone mass.

Strengths: They are accurate and can differentiate visceral and subcutaneous fat

Limitations: Equipment is extremely expensive and cannot be used for pregnant women and children due to ionizing radiation[73].

Though there are various methods available, in this study ,the most popular and convenient methods of measuring obesity such as Body mass index (BMI), Waist circumference(WC), Hip circumference(HC) and Waist-Hip circumference (WHR) are used to assess adiposity and body fat distribution.

CLASSIFICATION OF OBESITY

Table 1: WHO classification of obesity[74]

CLASSIFICATION	BMI
Underweight	< 18.5
Normal Range	18.5 – 24.9
Over weight	25.0 – 29.9
Preobese	25.0 -29.9
Obese class 1	30.0 – 34.9
Obese class ll	35.0 – 39.9
Obese class lll	40 or higher

Table 2: WHO classification of obesity for Asians[44]

CLASSIFICATION	BMI
Underweight	**< 18.5**
Normal Range	**18.5 – 22.9**
Over weight	**23.0 – 24.9**
Obese	**≥ 25**

ATHEROGENIC INDEX IN OBESITY

There is a close association between obesity and accelerated atherosclerosis and rate of cardiovascular death. Many plausible mechanisms have been proposed to explain how increase in adiposity can affect vessel walls of major and minor arteries. They include changes in plasma glucose, blood pressure, lipid and lipoprotein metabolism and systemic inflammation. Adipokines secreted from the adipose tissue directly influence vessel wall homeostasis that influence the function of arterial smooth muscle cells, endothelial cells and macrophages in the vessel wall.

Dyslipidemia, with elevated plasma triglycerides, total cholesterol and lipoproteins LDL-C, VLDL-C and low HDL-C is closely associated with obesity and plays an important role in the development of atherosclerosis and cardiovascular risks in obese individuals[75].

Dyslipidemia and atheroma formation

Accumulation of low density lipoprotein cholesterol (LDL-C) in the arteries recruits blood monocytes to atherosclerotic prone sites. These monocytes migrate to the subendothelial space and get differentiated to macrophages. Lipoproteins such as LDL-C,small dense LDL and lipoprotein (a) traverse the endothelium and get oxidized by the reactive oxygen species. These oxidized lipoproteins are taken up by the macrophage scavenger receptor and leads to the formation of foam cells, which is characteristic of early stage atherosclerosis. The lesion grows as the monocyte recruitment and lipoprotein influx continue, and develops into the fatty streak. Increased collagen synthesis by intimal smooth muscle cells of the blood vessels lead to formation of fibrous plaque or atheroma[75] .

Atherogenic Index of Plasma (AIP) have been shown as a strong predictor of atherosclerosis and coronary heart disease . AIP is associated with the plasma levels of atherogenic lipoproteins LDL, small dense LDL, lipoprotein (a) and reflects the relationship between protective (HDL) and atherogenic lipoprotein (LDL) . AIP is calculated according to the formula, log (TG/HDL-C)[76].

ROLE OF ADIPOKINES IN OBESITY

Adipose tissue has been proposed as a dynamic endocrine organ that secretes various type of chemo- mediators called adipokines. These adipokines and their balance have been proved to significantly modulate various metabolic processes. The function, secretion and the balance of adipokines

are disturbed in obesity and becomes the root cause of all the metabolic complications of obesity.

The expansion of visceral fat causes dysregulation of adipokine secretion which is accompanied by the cytokine production from the macrophages of the expanded adipose tissue results in decrease in insulin sensitivity. The low grade inflammation of the adipose tissue by the cytokines along with dyslipidemia become the risk factor for the development of atherosclerosis. Dysregulated adipokines also affect hunger, causing increased food intake through their receptors in the hypothalamus[77] .

LEPTIN

Leptin is the first discovered adipokine secreted from the adipocyte of white adipose tissue. The main function of leptin is to regulate lipid homeostasis in the body by stimulating storage of triglycerides in the adipose tissue rather than storage in non-fat tissues like skeletal muscles or pancreas. The receptors of leptin are present in hypothalamus, pancreatic islets, skeletal muscle, liver, kidney and lungs. Leptin acts as peripheral signal protein to the hypothalamus to regulate the appetite and energy metabolism. Leptin also is known to inhibit neurotransmitter neuropeptide Y, which is an appetite stimulator[77].

ADIPONECTIN

Adiponectin, secreted by the adipocytes is found to be negatively correlated with body mass index, plasma triglycerides and insulin resistance. There is low plasma levels of adiponectin in obesity. High fat diet causes hypertrophy of adipocytes and reduces the secretion of adiponectin.

Adiponectin regulates lipid metabolism by increasing transport and utilization of free fatty acids into the tissues thereby reducing lipid storage. It regulates appetite by acting as an appetite stimulator in the hypothalamus[77].

OMENTIN

Omentin is an adipokine secreted in abundance by the stroma of blood vessels in the visceral adipose tissue.It regulates glucose metabolism by increasing the insulin sensitivity in the cells but does not show insulin-mimetic effect .The major circulating isoform of omentin, omentin-1 is associated with appetite regulation and has orexigenic effect.It increases food intake and body weight and positively correlates with the degree of obesity and insulin resistance[77].

VASPIN

Vaspin is a protein that structurally belong to the family of serine protease inhibitors, called serpin. Vaspin is produced by both subcutaneous and visceral adipose tissue. The serum concentrations of vaspin is related to food intake and had diurnal variation. The peak concentration is at early morning before breakfast and comes to basal level 2 hours after breakfast. Though vaspin serum concentrations is positively correlated with obesity, insulin resistance and type 2 diabetes ,the exact mechanism of metabolic regulations are yet to be elucidated[77].

RESISTIN

The mRNA expression of resistin is found to be at a higher level in monocytes and macrophages of the adipose tissue rather than adipocytes. The plasma levels of resistin are positively correlated with the inflammatory

markers like C-Reactive Protein (CRP), Tumour Necrosis Factor (TNF -2), Interleukin (IL-6) in obese individuals hypothezing the link between resistin and inflammatory sequence of metabolic syndrome and insulin resistance. The serum resistin levels are found to be increased along with inflammatory markers in obesity, type 2 diabetes and insulin resistance suggesting the fact that the production of resistin is secondarily increased in obesity related inflammatory conditions in humans[77].

ADIPOCYTE FATTY ACID BINDING PROTEIN (AFABP)

Adipocyte Fatty Acid Binding Protein (AFABP) belongs to the family of mammalian intracellular Fatty Acid-Binding Proteins (FABP). Increased levels of AFABP are involved in the transport of fatty acids and affects plasma lipids resulting in insulin resistance, metabolic syndrome and development of atherosclerosis. Plasma levels of AFABP in humans are positively correlated with the degree of obesity, high blood pressure, waist circumference, and altered lipid profile. High levels of AFABP was found to worsen the prognosis of cardio metabolic risk and is considered as a marker of metabolic syndrome[77].

TNF-α

TNF-α, which is produced by the macrophages of the adipose tissue, exerts direct effect on the insulin signaling cascade. This adipokine induces phosphorylation of the insulin receptor, preventing interaction of insulin with the insulin receptor. It increases the activity of hormone-sensitive lipase in the adipose tissue and releases free fatty acids into circulation, which promotes

insulin resistance In humans, the plasma levels of TNF-α positively correlates with obesity and insulin resistance[77].

INTERLEUKIN-6 (IL-6)

Interleukin-6 (IL-6) is pro-inflammatory cytokine secreted in the adipose tissue. It increases lipolysis and free fatty acids release in circulation and also increases hepatic synthesis of fatty acids and cholesterol and is associated with obesity related triglyceridemia, in humans. IL-6 is positively correlated with Obesity, Dyslipidemia And Insulin Resistance[77].

INTERLEUKIN-1 β (IL-1 β)

Interleukin-1β (IL-1β) is also a pro-inflammatory cytokine secreted by the macrophages of adipose tissue. IL-1β reduces the expression of insulin receptor substrate 1 (IRS-1) at the level of transcription impairing the insulin signaling pathway. Its elevated levels are seen in insulin resistance obesity and metabolic syndrome[77].

VISFATIN

In this study, serum levels of this adipokine, visfatin is correlated with BMI and adiposity. Visfatin**,** a recently discovered adipokine is predominantly expressed in the visceral adipose tissue. But,later it was found that this protein was previously known in the name PBEF (pre-B-cell colony enhancing factor) as a modulator of B-cell differentiation. This adipokine had also been known previously as an intracellular enzyme , nicotinamide 5-phosphoribosyl-1-pyrophosphate transferase (NAMPT) that catalyses the conversion of

nicotinamide to nicotinamide mononucleotide[24]. Fukuhara in 2005 found this novel cytokine and named it as Visfatin[21].

Visfatin regulates the metabolism of lipids and carbohydrates mainly by its ability to bind to insulin receptors and activate the insulin signaling cascade by phosphorylating IRS-1/-2, insulin receptor substrate molecule. Its action is similar to the action of insulin, stimulating glucose uptake into cells and lipogenesis[30].

Though visfatin has insulin-mimetic properties, it cannot replace insulin in the regulation of glucose metabolism as it binds to a different site in the insulin receptor distinct from the insulin binding site.The serum concentration of visfatin is only one-tenth of insulin and is not regulated by plasma glucose level or food intake[30].

In addition to insulin-mimetic action,it mediates differentiation of adipocytes and lipogenesis in the visceral adipose tissue resulting in the extended ability of visceral fat to absorb and store larger amount of lipids. Many studies have shown that the plasma levels of Visfatin was found to be positively correlated with obesity. Plasma levesls of Visfatin was previously reported to be increased in obesity and other studies have shown that there was elevated Visfatin concentrations type 2 diabetes patients[30]. Several studies have shown conflicting results regarding visfatin concentrations and insulin levels and more studies are needed to establish clinical relevance between visfatin and glucose metabolism[78,79]. PBEF/visfatin has also been shown as proinflammatory cytokine, as its serum/plasma levels are increased in various inflammatory disorders[80].

It was demonstrated by Fukuhara that plasma Visfatin levels strongly correlate with the quantity of visceral adipose tissue assessed by computed tomography[21]. Conflicting results were obtained in different studies assessing correlations between Visfatin, anthropometric parameters and Athergenic index.

Hence in this study, correlation between serum Visfatin concentration and body fat distribution with anthropometric measurments -Waist Circumference (WC),Waist Hip Ratio (WHR) and correlation with Atherogenic index was elucidated.

AIMS AND OBJECTIVES

AIM

To evaluate the serum visfatin levels in young obese adults compared with non-obese individuals.

OBJECTIVES

1. To establish the relationship between serum visfatin levels and selected anthropometric parameters (BMI, Waist circumference, Hip circumference, Waist-Hip Ratio) in young obese individuals.

2. To study the correlation between serum visfatin levels and the lipid parameters of young obese individuals.

3. To explore the association between serum visfatin levels and atherogenic index in young obese adults.

MATERIALS AND METHODS

STUDY DESIGN **:** Cross – sectional study

PLACE OF STUDY **:** Department of Biochemistry, Chennai Medical College Hospital & Research Centre, Irungalur, Trichy.

PERIOD OF STUDY **:** May 2016 – May 2017

SAMPLE SIZE **:** 60 Cases and 30 Controls

(Cases – obese individuals)

(Controls – non obese individuals)

AGE **:** 19 – 35 years (young adults)

SEX **:** Both males and females

GEOGRAPHICAL DISTRIBUTION **:** Both urban and rural areas

ETHICAL CONSIDERATIONS:

The necessary approval was obtained to conduct the study from the Ethical Committee Board, Chennai Medical College Hospital and Research centre, Irungalur, Trichy. Patients were given explanation about the purpose of study and informed written consent was obtained and the confidentiality of their results was assured.

SELECTION OF CASES AND CONTROLS:

CASES: Sixty obese young adults, came for master health check up to the Chennai Medical College Hospital and Research Centre, were selected as cases. Both

males and females in the age group of 19 – 35 years, were selected and obesity was defined as those with BMI ≥ 25 kg/m^2, set by WHO for Asians[44].

Inclusion criteria:

Obese young adults of both the sexes with BMI ≥ 25 kg/m^2 were included in the study.

Exclusion criteria:

1. Obese adults of more than 35 years and less than 19 years were excluded in the study.

2. Obese young adults with co-morbid illnesses like Hypothyroidism, Diabetes Hypertension, Pregnant women and women on Oral Contraceptive Pills were excluded from the study.

CONTROLS:

Age and sex matched controls, with BMI ≤ 25 kg/m^2, who attended Master Health Check-up Department, in the Chennai Medical College Hospital and Research Centre, were taken as controls.

STUDY PROTOCOL:

After obtaining the informed written consent, all patients were subjected to detailed history taking and clinical examination.

HISTORY:

A detailed history was elicited for

- Thyroid disorders
- Diabetes

- Hypertension
- Concomitant drug intake – oral contraceptive pills, Anti-thyroid drugs.
- In females – if they are pregnant or in Amenorrhea.

CLINICAL EXAMINATION

Pulse, Systolic and Diastolic Blood Pressure were recorded.

Blood pressure was recorded in the sitting position in the right arm using mercury sphygmomanometer .

SYSTEMIC EXAMINATION

Systemic Examination included examination of

Cardiovascular system – Ausculcated for S_1 and S_2 and murmurs if any.

Respiratory system – Auscultated on both sides of chest for normal Vesicular Breath Sounds and for Added sounds if any.

Abdomen – Palpated for any organomegaly and observed for any abdomen Tenderness.

Central Nervous System – Examined for normal reflexes and for any focal neurological Deficits.

ANTHROPOMETRIC MEASUREMENTS

Height, Weight, Hip circumference and Waist circumference were measured . BMI and Waist-Hip ratio were calculated from the above parameters.

1. HEIGHT :

Height (in centimeters) was measured using a Stadiometer. The individual was asked to stand upright without shoes with his/her back against the vertical back board, heels together and eyes directed forward

2. WEIGHT:

Weight(in kilograms) was measured with an electronic weighing scale that was kept on a firm horizontal flat surface. Subjects were asked to wear light clothing, and weight was recorded to the nearest 0.5 kg.

3. WAIST CIRCUMFERENCE (WC)

Waist circumference (in centimeters) was measured using a non-stretchable measuring tape. Waist circumference was measured at the smallest horizontal girth between the costal margins and the iliac crest at the end of expiration.

4. HIP CIRCUMFERENCE (HC)

Hip circumference (in centimeters) is measured at the widest diameter of gluteus maximus .

5. BODY MASS INDEX (BMI)

Body mass index (BMI) was calculated using the formula

Weight (kg) / Height (m^2).

6. WAIST – HIP RATIO (WHR)

Waist – hip ratio was calculated by dividing waist circumference in cm by Hip circumference in cm.

INVESTIGATIONS:

1. Fasting Blood Glucose
2. Fasting serum lipid profile

    - Total cholesterol
    - Triglycerides
    - Low density lipoprotein -cholesterol
    - High density lipoprotein- cholesterol

3. Serum Visfatin

COLLECTION OF VENOUS BLOOD

- Informed consent was obtained for each individual from cases and control groups prior to the study.
- After 8- 12 hours of fasting ,the subjects were made to sit for 10 minutes and then,5 ml of venous blood was collected by venous puncture in the medial cubital vein in the anterior cubital fossa of forearm, under strict aseptic precaution .
- The venous blood was collected in clot activator coated polypropylene tubes after removal of needle from the syringe.
- Blood was centrifuged at 3500 rpm for 10 minutes and serum was separated.
- The serum that was separated was used for the Quantitative analysis of various parameters

ESTIMATED PARAMETERS

1. Fasting Serum Glucose - Glucose Oxidase – Peroxidase Method

2. Serum Lipid Profile

- Serum Total Cholesterol - Cholesterol Oxidase / Peroxidase Method
- Serum Triglycerides - Glycerol 3 – Phosphate Oxidase Method
- Serum LDL-C (Low density Lipoprotein Cholesterol) -- Direct enzymatic method
- Serum HDL-C (High density Lipoprotein Cholesterol) - Direct enzymatic method
- Serum VLDL -C(High density Lipoprotein Cholesterol)-calculated using the formula :Triglycerides /5

3 . Serum Visfatin – Enzyme Linked Immunosorbant Assay (ELISA) Method

CALCULATED PARAMETERS

1. Body Mass Index (BMI) : $\frac{\text{Weight (kg)}}{\text{Height } (m^2).}$

2. Waist – Hip ratio (WHR) : $\frac{\text{Waist circumference in cm}}{\text{Hip circumference in cm}}$

3. Very Low Density Lipoprotein : TGL / 5

DEFINITIONS

Hypertension

Hypertension was defined by systolic BP ≥140 mmHg and/or a diastolic BP ≥90 mmHg or use of antihypertensive medications .

Diabetes Mellitus

Diabetes Mellitus was defined by the fulfillment of criteria laid down by the ADA(American Diabetic Association) criteria of fasting glucose of ≥126 mg/dl ,postprandial glucose of ≥ 200 or by physician diagnosis of diabetes and current use of medications for diabetes (insulin or oral hypoglycaemic agents)

Obesity

Obesity was defined as a BMI ≥ 25 kg/m^2 for both genders (based on the WHO Asia Pacific Guidelines) [12]

QUANTITATIVE ESTIMATION OF FASTING BLOOD GLUCOSE:

METHODOLOGY:

GLUCOSE OXIDASE – PEROXIDASE METHOD **(END POINT METHOD)**

PRINCIPLE:

Glucose present in serum / plasma is oxidized by the enzyme glucose oxidase (GOD) to gluconic acid with the liberation of hydrogen peroxide, which is converted to water and nascent oxygen by the enzyme peroxidase (POD).

4-Amino antipyrine,an oxygen acceptor takes up the oxygen and together with phenol forms a pink colored chromogen which can be measured at 505 nm.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{phenol} + \text{4 – aminoantipyrine} \xrightarrow{\text{POD}} \text{Red quinoneneimine}$$

GLUCOSE REAGENTS:

Phosphate buffer(ph 7.5) : 0.1 mol/L

4 –Aminoantipyrine : 5.0 mmol/L

Peroxidase : >1.5 KU/L

Glucose oxidase : >15 KU/L

Phenol : 5.0 mmol/L

Glucose standard concentration : 100 mg/dl

ASSAY PROCEDURE : (FULLY AUTOMATED ANALYZER)

PROCEDURE:

The Glucose reagent are brought to room temperature (25^0 c) . Pipette the reagent into test tubes and labeled them as Blank(B), Standard(S) and Test (T) as follows

S.NO	REAGENT	BLANK	STANDARD	TEST
1	GLUCOSE REAGENT	1.0 ML	1.0 ML	1.0 ML
2	GLUCOSE STANDARD	-	10µl	-
3	SERUM	-	-	10µl

Reaction temperature at 37^0c.

Mix well and read absorbance of Standard (S) and Test (T) against blank (B) at 505 nm or with green filter (500 – 540 nm).

CALCULATION OF RESULTS

$$\text{Glucose conc (mg/dl)} = \frac{\Delta \text{ Abs for Test}}{\Delta \text{ Abs for Standard}} \times 100$$

Reference value:

Serum/ plasma (fasting) : 70 – 100 mg/dl

Serum / plasma (post prandial) : <140 mg/dl

LIPID PROFILE

QUANTITATIVE ESTIMATION OF SERUM TOTAL CHOLESTEROL

METHODOLOGY:

Cholesterol oxidase / Peroxidase method

PRINCIPLE OF THE METHOD

The enzyme cholesterol esterase is used to hydrolyze the cholesterol esters present in the serum to free cholesterol and free fatty acids. The enzyme cholesterol oxidase in the presence of oxygen oxidizes the cholesterol to cholestenone and hydrogen peroxide. Hydrogen peroxide oxidizes phenol and 4-aminoantipyrine to produce red color that can be measured spectrophotometrically.

Cholesterol esters + H_2O $\xrightarrow{\text{Chol .esterase}}$ Cholesterol +free fatty acids

Cholesterol + O2+ H_2O + H_2O_2 $\xrightarrow{\text{Chol .oxidase}}$ Cholest-4-en-3-one + H_2O_2

2 H_2O_2 + Phenol + 4-Aminoantipyrene $\xrightarrow{\text{Peroxidase}}$ Quinone mine dye +4 H_2O

The intensity of the color formed is proportional to the cholesterol concentration in the serum.

CHOLESTEROL REAGENTS:

Pipes	: 35 mmol/l
Sodium cholate	: 0.5 mmol/l
Phenol	: 26mmol/L
4-Amino antipyrene(4-AA)	: 0.5mmol/L
Cholesterol esterase(CHE)	: >0.2 U/ml
Cholesterol oxidase(CHOD)	: > 0.1U/ml
Peroxidase (POD)	: > 0.8U/ml
PH	: 7.0

The Cholesterol Standard concentration (5 ml) : 200mg/dl

The reagents were stored at 2^0 -8 ^{0}c.

PREPARATION OF WORKING SOLUTION :

The reagents are allowed to attain room temperature.

PROCEDURE :

The sample and the working solution are brought to room temperature prior to use. 3 test tubes were taken and labelled them as Blank(B),Standard(S) ,Test(T) . 1ml of working reagent was added to 3 test tubes. 10 µl of standard was added to test tube labelled 'T' and 10 µl of standard was added to test tube labelled 'S'.It was mixed and incubated for 10 minutes at room temperature.

	Blank	**Standard**	**Test**
Distilled water	**10µL**	**-**	**-**
Reagent	**1ml**	**1ml**	**1ml**
Standard	**-**	**10 µL**	**-**
Sample	**-**	**-**	**10 µL**

CALCULATIONS:

$$\frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 200 = \text{Sample concentration (mg/dl)}$$

LINEARITY:

This method is used to measure serum Total Cholesterol levels upto 1000mg/dl.

REFERENCE VALUES:

SERUM TOTAL CHOLESTEROL:

Desirable : up to 200 mg/dl

Borderline : 200 – 239 mg/dl

High : > 240 mg/dl

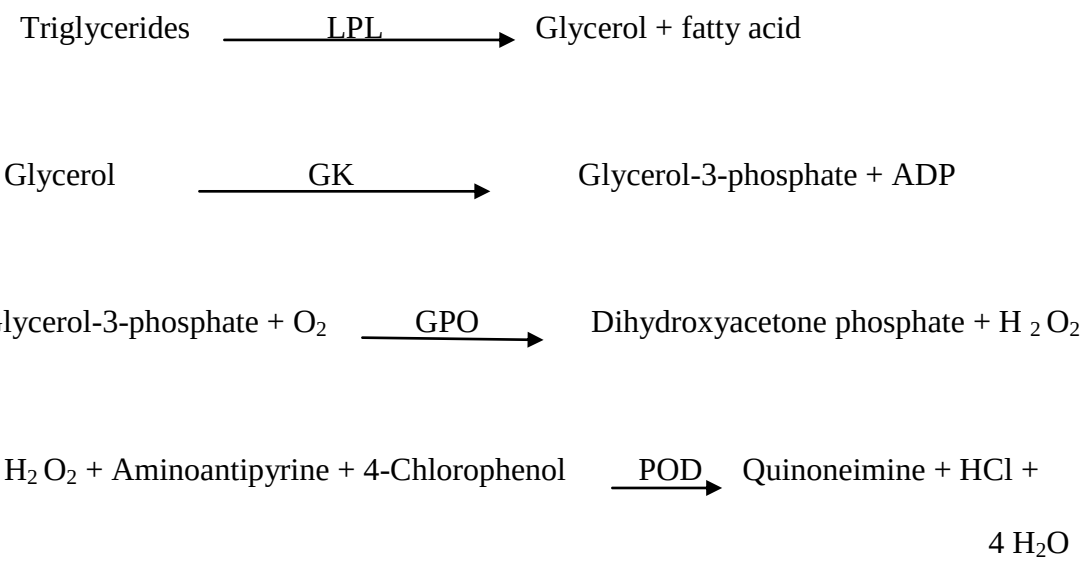
QUANTITATIVE ESTIMATION OF SERUM TRIGLYCERIDES

METHODOLOGY:

Colorimetric enzymatic test using Glycerol-3-phosphate-oxidase (GPO)

PRINCIPLE OF THE METHOD

Determination of triglycerides after enzymatic splitting with lipoprotein lipase. Indicator is quinoneimine which is generated from 4-aminoantipyrine and 4-chlorophenol by hydrogen peroxide under the catalytic action of peroxidase.



REAGENTS:

Components and Concentrations:

Good's buffer pH 7.2	: 50 mmol/L
4-Chlorophenol	: 4 mmol/L
ATP	: 2 mmol/L
Mg^{2+}	: 15 mmol/L
Glycerol kinase (GK)	: ≥ 0.4 kU/L
Peroxidase (POD)	: ≥ 2 kU/L
Lipoprotein lipase (LPL)	: ≥ 2 kU/L
4-Aminoantipyrine	: 0.5 mmol/L
Glycerol-3-phosphate-oxidase (GPO)	: ≥0.5 kU/L

The concentration of the Standard : Triglycerides 200 mg/dl

The reagents were stored at $2^0 – 8\ ^0$c

PREPARATION OF WORKING SOLUTION :

The reagents are allowed to attain room temperature.

PROCEDURE :

The sample and the working solution are brought to room temperature prior to use.3 test tubes were taken and labelled them as Blank(B),Standard(S) ,Test(T) . 1ml of working reagent was added to 3 test tubes. 10 µl of standard was added to test tube labelled ‘T’ and 10 µl of standard was added to test tube labelled ‘S’. It was mixed and incubated for 10 minutes at room temperature.

	Blank	Standard	Test
Distilled water	**10µL**	**-**	**-**
Reagent	**1ml**	**1ml**	**1ml**
Standard	**-**	**10 µL**	**-**
Sample	**-**	**-**	**10 µL**

CALCULATIONS:

$$\frac{\textbf{Sample absorbance}}{\textbf{Standard absorbance}} \textbf{ X 200 = sample concentration (mg/dl)}$$

To correct for free glycerol,subtract 10 mg/dl from the triglycerides value calculated above.

LINEARITY:

This method is used to measure serum Triglycerides concentration within a measuring range from 2 – 1000 mg/dl.

REFERENCE VALUES:

SERUM TRIGLYCERIDES:

Desirable : < 200 mg/dl

Borderline : 200 – 400 mg/dl

High : > 400 mg/dl

QUANTITATIVE ESTIMATION OF SERUM HIGH DENSITY LIPOPROTEIN CHOLESTEROL(HDL – C)

METHODOLOGY:

Direct enzymatic method

PRINCIPLE OF THE METHOD

Antibodies against human lipoproteins are used to form antigen-antibody complexes with LDL, VLDL and chylomicrons in a way that only HDL-cholesterol is selectively determined by an enzymatic cholesterol measurement.

LDL, VLDL, Chylomicrons $\xrightarrow{\text{Anti-human β-lipoprotein antibodies}}$ Antigen-antibody complexes + HDL

HDL-cholesterol + H_2O + O2 $\xrightarrow{\text{CHE \& CHO}}$ Cholesterol-3-one + fatty acid + H_2O_2

H_2O_2 + F-DAOS + 4-Aminoantipyrine $\xrightarrow{\text{POD}}$ Blue complex + H_2O

REAGENTS

Components and Concentrations

Reagent 1:

Good's buffer	pH 7.0	: 25 mmol/L
4-Aminoantipyrine		: 0.75 mmol/L
Peroxidase (POD)		: 2 kU/L
Ascorbate oxidase		: 2.25 kU/L

Anti-human β-lipoprotein antibody (sheep)

Reagent 2:

Good's buffer pH 7.0	: 30 mmol/L
Cholesterol esterase (CHE)	: 4 kU/L
Cholesterol oxidase (CHO)	: 20 kU/L
N-Ethyl-N-(2-hydroxy-3-sulfopropyl) - 3,5-dimethoxy-4- fluoroaniline, sodium salt (F-DAOS)	: 0.8 mmol/L

Calibrator : HDL – cholesterol : 50.6 mg/dl

Both the reagents were stored at 2^0 -8 ^{0}c .

PREPARATION OF WORKING SOLUTION :

The reagents are allowed to attain room temperature.

PROCEDURE :

The sample and the working solution were brought to room temperature prior to use. 3 test tubes were taken and labelled them as Blank (B), Standard (S) , Test (T) . 240 µl of working reagent was added to 3 test tubes. 2.4 µl of sample was added to test tube labelled 'T' and 2.4 µl of standard was added to test tube labelled 'C.It was mixed and incubated for 5 minutes at room temperature. The absorbance A_1 was read, then 60 µl of working reagent 2 was added to 3 test tubes. It was mixed and incubated for 5 minutes at room temperature. The absorbance A_2 was read.

	BLANK	CALIBRATOR	TEST
CALIBRATOR	-	**2.4** µl	-
SAMPLE	-	-	**2.4** µl
DISTILLED WATER	**2.4** µl	-	-
REAGENT 1	**240** µl	**240** µl	**240** µl

Mixed, incubated for 5 min, at 37^0 c , absorbance (A_1), was read then added:

REAGENT 2	**60** µl	**60** µl	**60** µl

ΔA = (A2 – A1) sample or calibrator - (A2 – A1) blank

CALCULATIONS :

$$\frac{\Delta A \text{ sample}}{\Delta A \text{ Calibrator}} \times \text{conc. Calib} = \text{sample concentration (mg/dl)}$$

LINEARITY:

This method determines HDL-C concentrations within a measuring range from 1 – 180mg/dl.

REFERENCE VALUES :

SERUM HDL – C: Males - 30 – 60 mg/dl

Females – 35 – 75 mg/dl

QUANTITATIVE ESTIMATION OF SERUM LOW DENSITY LIPOPROTEIN CHOLESTEROL

METHODOLOGY

ENZYMATIC METHOD

PRINCIPLE OF THE METHOD

LDL is selectively protected while non-LDL-lipoproteins are enzymatically processed. In a second step, LDL is released and LDL-cholesterol selectively determined in a color producing enzymatic reaction.

1) LDL + reagent 1 $\longrightarrow$ Protected LDL

HDL, VLDL, Chylomicrons $\xrightarrow{\text{CHE \& CHO}}$ Cholestenone + H_2O_2

H_2O_2 $\xrightarrow{\text{Catalase}}$ H2O

2) Protected LDL + reagent 2 $\longrightarrow$ LDL

LDL-C CHE & CHO $\longrightarrow$ Cholestenone + H_2O_2

H_2O_2 + 4-Aminoantipyrine + H-DAOS $\xrightarrow{\text{POD}}$ Color

REAGENTS :

Components and Concentrations

Reagents 1:

Good's buffer pH 6.8 : 20 mmol/L

Cholesterol esterase (CHE) : ≥ 2.5 kU/L

Cholesterol oxidase (CHO) : ≥ 2.5 kU/L

N-(2-hydroxy-3-sulfopropyl)-

3,5-dimethoxyaniline (H-DAOS) : 0.5 mmol/L

Catalase : ≥ 500 kU/L

Reagent 2:

Good's buffer	pH 7.0	: 25 mmol/L
4-Aminoantipyrine		: 3.4 mmol/L
Peroxidase (POD)		: ≥ 15 kU/L

The reagents were stored at 2 – 8 °C

	Blank	**Sample or calibrator**
Sample or calibrator	-	3.0 KL
Dist. Water	3.0 KL	-
Reagent 1	280 KL	280 KL

Mixed, incubated for 5 min. at 37 °C, absorbance (A1) was read, then added:

Reagent 2	70 KL	70 KL

Mixed, incubated for 5 min. at 37 °C, absorbance (A2) was read.

ΔA = (A2 – A1) sample or calibrator - (A2 – A1) blank

Calculation

With calibrator

$$\text{LDL –C(mg/dl)} = \frac{\Delta \text{ A sample}}{\Delta \text{ A calibrator}} \times \text{Conc. Cal. [mg / dL]}$$

REFERENCE VALUES :

SERUM LDL-C:

Desirable : ≤130mg/dl

Borderline high risk : 130 – 160 mg/dl

High risk : > 160 mg/dl

LINEARITY

This method determines LDL-C concentrations within a measuring range from 1 - 400 mg/dL (0.03 – 10.3 mmol/L).

QUANTITATIVE ESTIMATION OF SERUM VISFATIN

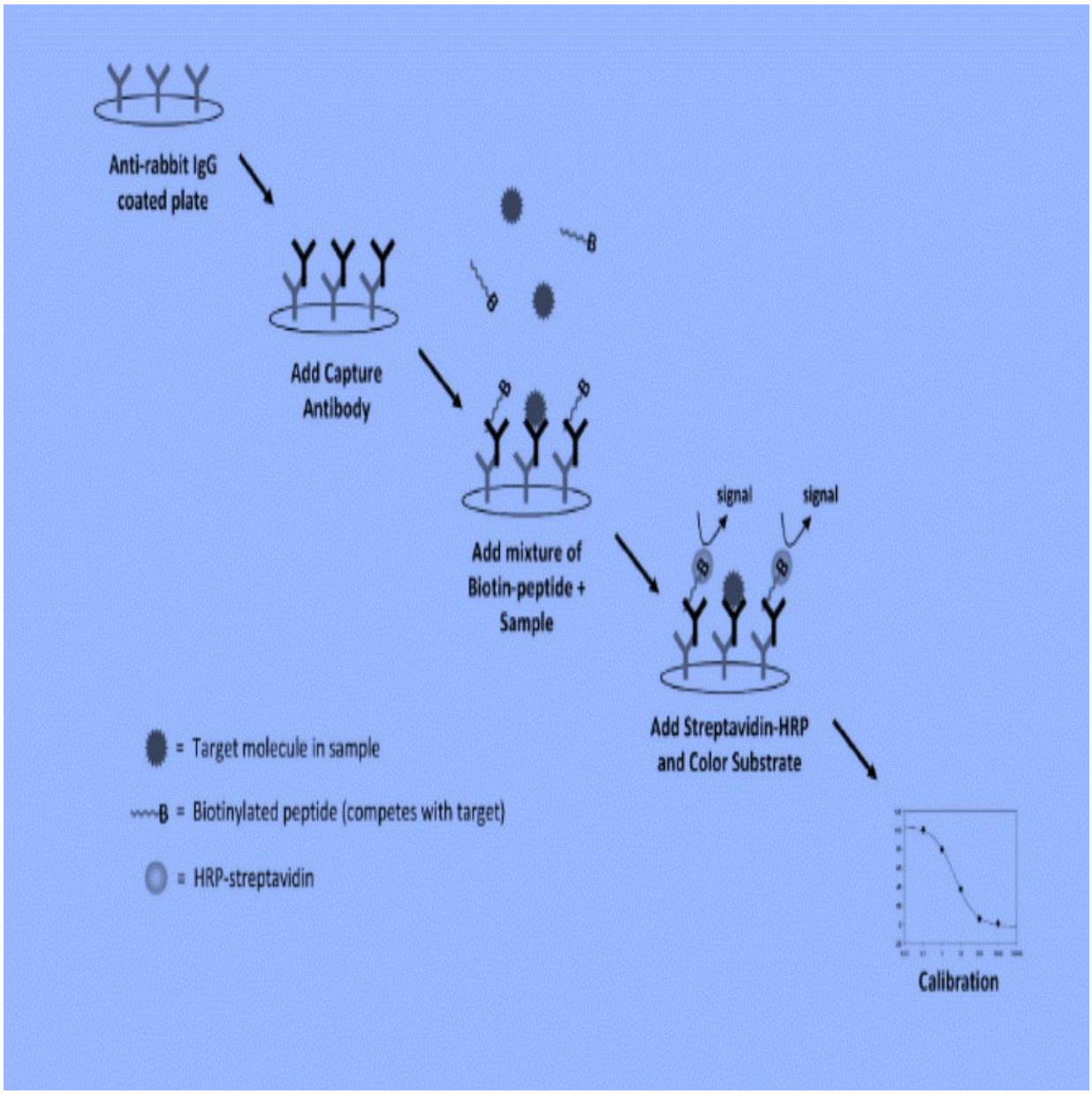
METHODOLOGY

ENZYME LINKED IMMUNOSORBANT ASSAY **(ELISA)**

PRINCIPLE OF THE ASSAY

The VISFATIN (Human) ELISA Kit is an in vitro quantitative assay for detecting Visfatin peptide based on the principle of Competitive Enzyme Immunoassay. The microplate in the kit is pre-coated with anti-rabbit secondary antibody. After a blocking step and incubation of the plate with anti-Visfatin antibody, both biotinylated Visfatin peptide and peptide standard or targeted peptide in samples interacts competitively with the Visfatin antibody. Uncompeted (bound) biotinylated Visfatin peptide then interacts with Streptavidin-horseradish peroxidase (SA-HRP), which catalyzes a color development reaction. The intensity of colorimetric signal is directly proportional to the amount of biotinylated peptide-SA-

HRP complex and inversely proportional to the amount of Visfatin peptide in the standard or samples. This is due to the competitive binding to Visfatin antibody between biotinylated Visfatin peptide and peptides in standard or samples. A standard curve of known concentration of Visfatin peptide can be established and the concentration of Visfatin peptide in the samples can be calculated accordingly.



MATERIALS :

Component	Amount
Visfatin Microplate (Item A): 96 wells coated with secondary antibody.	96(12 x8)wells
Wash Buffer Concentrate (20x) (Item B)	25 mL
Lyophilized standard Visfatin Peptide (Item C)	2 vials
Lyophilized anti-Visfatin polyclonal antibody (Item N)	2 vials
1X Assay Diluent E(Item R): Diluent for both standards and samples including serum or plasma, cell culture media or other sample types.	25 mL x 2
Lyophilized biotinylated Visfatin peptide, (Item F)	2 vials
HRP-Streptavidin concentrate (Item G): 100x concentrated HRP-conjugated Streptavidin.	600 µL
Lyophilized positive control (Item M)	1 vial
TMB One-Step Substrate Reagent (Item H): 3, 3', 5, 5'- tetramethylbenzidine (TMB) in buffered solution.	12 mL
Stop Solution (Item I): 0.2 M sulfuric acid.	8 mL

Storage Instructions

- Standard, Biotinylated Visfatin peptide, and Positive Control should be stored at -20°C after arrival. Avoid multiple freeze-thaws.
- Opened Microplate Wells and antibody (Item N) may be stored for up to 1 month at 2° to 8°C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.
- If stored in this manner, Ray Bio warranties this kit for 6 months from the date of shipment

ASSAY PROTOCOL

Reagent Preparation

1. Kit reagents were kept on ice during reagent preparation steps. Equilibrate plate to room temperature before opening the sealed pouch.

2. Briefly centrifuged the Visfatin Antibody vial (Item N) and reconstitute with 5 μL of ddH2O before use. Added 50 μL of 1x Assay Diluent E into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently.

3. The antibody concentrate was diluted 100-fold with 1x Assay Diluent E. This is the anti-Visfatin antibody working solution, which will be used in step 2 of the Assay Procedure.

4. Briefly centrifugde the vial of biotinylated Visfatin peptide (Item F) and reconstitute with 20 μL of ddH2O before use. Added 5 μL of Item F to 5 mL 1X Assay Diluent E. Pipette up and down to mix gently. The final concentration of

biotinylated Visfatin should be 10 ng/mL. This solution will only be used as the diluent in step 5 of Reagent Preparation.

5. **Preparation of Standards:**

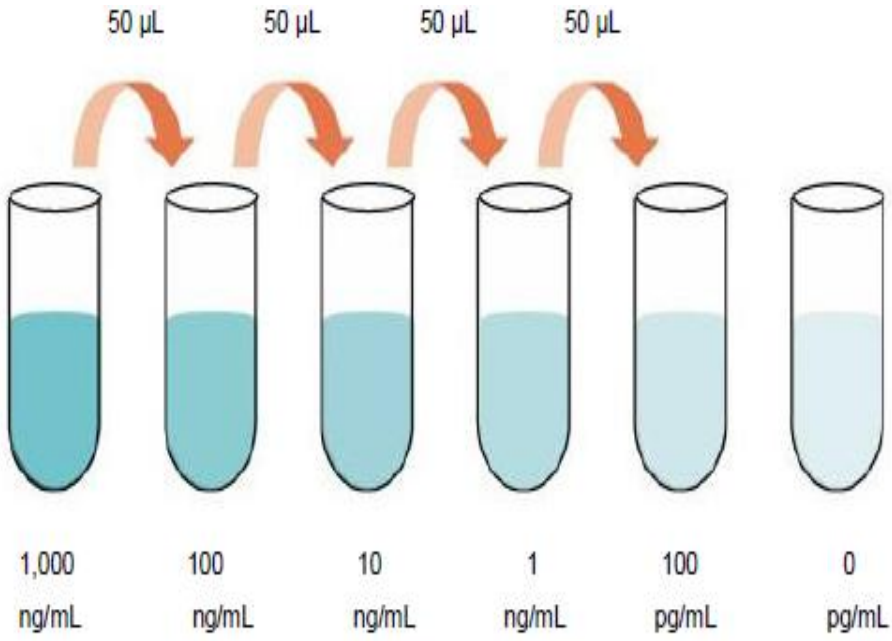
Labelled 6 microtubes with the following concentrations: 1000 ng/mL, 100 ng/mL, 10 ng/mL, 1 ng/mL, 100 pg/mL and 0 pg/mL. Pipette 450 µL of biotinylated Visfatin solution into each tube, except for the 1000 ng/mL (leave this one empty). It was made sure the concentration of biotinylated Visfatin is 10 ng/mL in all standards.

a. Briefly centrifuged the vial of standard Visfatin peptide (Item C) and reconstitute with 10 µLof dd H2O. In the tube labeled 1000 ng/mL, pipetted 8 µLof Item C and 792 µL of 10 ng/mL biotinylated Visfatin solution. This is the Visfatin stock solution (1000 ng/mL Visfatin, 10 ng/mL biotinylated Visfatin). Mix thoroughly. This solution served as the first standard.

b. To make the 100 ng/mLstandard, pipetted 50 µLof Visfatin stock solution the tube labeled 100 ng/mL. Mixed thoroughly.

c. Repeated this step with each successive concentration, preparing a dilution series as shown in the illustration below. Each time, used 450 µLof biotinylated Visfatin and 50 µL of the prior concentration until 100 pg/mLis reached. Mixed each tube thoroughly before the next transfer.

d. The final tube (0 pg/mLVisfatin, 10 ng/mLbiotinylated Visfatin) serves as the zero standard (or total binding).



6. Prepared a 10-fold dilution of Item F. To do this, add 2 µL of Item F to 18 µL of the 1X Assay Diluent. This solution was used in steps 7 and 9.

7**. Positive Control Preparation**: Briefly centrifuged the positive control vial and reconstitute with 100 µLof ddH2O before use (Item M). To the tube of Item M, added 101 µL1x Assay Diluent E. Also add 2µLof 10-fold diluted Item F (prepared in step 6) to the tube. This is a 2-fold dilution of the positive control. Mixed thoroughly. The positive control is a cell culture medium sample that is meant to be a system control (to verify that the detection and kit components are working). The result OD was not used in any calculations. It may be diluted further if desired, but was made sure the final concentration of biotinylated Visfatin is 10 ng/mL.

8. If Item B (20X Wash Concentrate) contains visible crystals, warm to room temperature and mixed gently until dissolved. Diluted 20mL of Wash Buffer Concentrate into deionized or distilled water to yield 400mL of 1x Wash Buffer.

9. **Sample Preparation:** Used 1X Assay Diluent E+ biotinylated Visfatin to dilute samples, including serum/plasma, cell culture medium and other sample types. It is very important to make sure the final concentration of the biotinylated Visfatin is 10 ng/mL in every sample. EXAMPLE: to make a 4-fold dilution of sample, mix together 2.5 µL of 10-fold diluted Item F (prepared in step 6), 185 µL of 1XAssay Diluent E, and 62.5 µL of the sample; mixed gently. The total volume was 250 µL, enough for duplicate wells on the microplate.

10. Briefly centrifuged the HRP-Streptavidin vial (Item G) before use. The HRP-Streptavidin concentrate was diluted 100-fold with 1X Assay Diluent E.

Assay Procedure

1. Kit reagents were kept on ice during reagent preparation steps. It was recommended that all standards and samples be run in duplicate.

2. Added 100 µL anti-Visfatin antibody (see Reagent Preparation step 3) to each well. Incubated for 1.5 hours at room temperature with gentle shaking (1-2 cycles/sec)

3. Discarded the solution and wash wells 4 times with 1x Wash Buffer (200-300 µL each), Washing was done with a multichannel pipette or an automated plate washer. Complete removal of liquid at each step was done for good assay performance. After the last wash, removed any remaining Wash Buffer by aspirating or decanting. Inverted the plate and bloted it against clean paper towels.

4. Added 100 µL of each standard, positive control and sample into appropriate wells. Included a blank well. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking (1-2 cycles/sec) or overnight at 4°C.

5. Discarded the solution and washed 4 times as directed in Step 3.

6. Added 100 µL of prepared HRP-Streptavidin solution to each well. Incubated for 45 minutes with gentle shaking at room temperature. It was recommended that incubation time should not be shorter or longer than 45 minutes.

7. Discarded the solution and wash 4 times as directed in Step 3.

8. Added 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubated for 30 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec).

9. Added 50 µL of Stop Solution (Item I) to each well. Read absorbances at 450 nm immediately.

Summary

1. Prepared all reagents, samples and standards as instructed.

2. Added 100 µL anti-Visfatin antibody to each well. Incubate 1.5 hours at room temperature or overnight at 4°C.

3. Added 100 µL standard or sample to each well. Incubate 2.5 hours at room temperature or overnight at 4°C.

4. Added 100 µL prepared streptavidin solution. Incubate 45 minutes at room temperature.

5. Added 100 µL TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.

6. Added 50 µL Stop Solution to each well. Read at 450 nm immediately .

Data Analysis

Calculation of Results

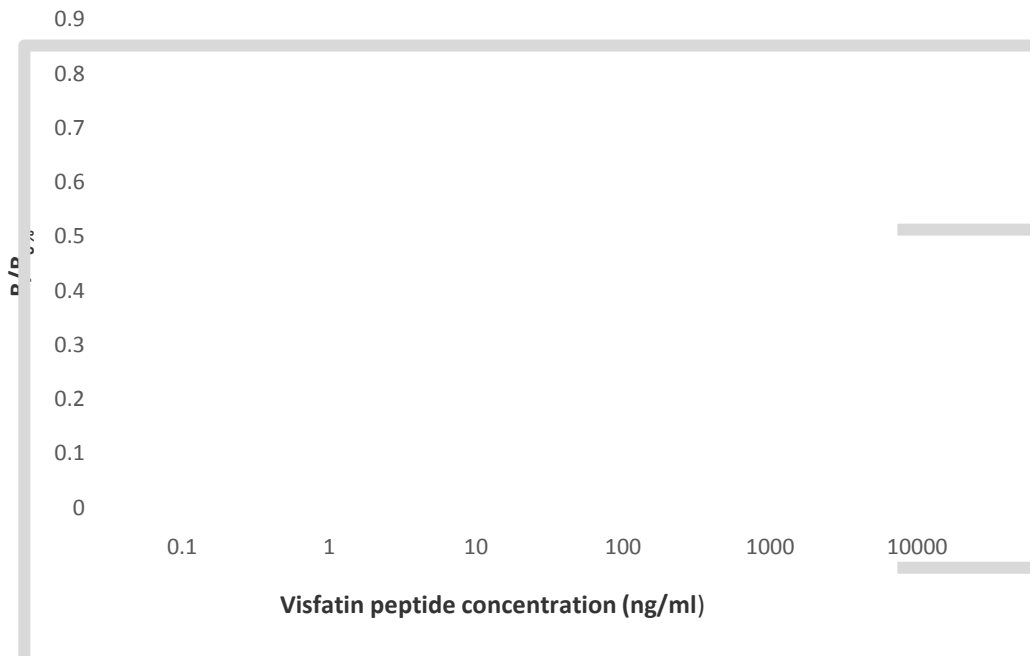
Calculated the mean absorbance for each set of duplicate standards, controls and samples, and subtracted the blank optical density. Plotted the standard curve using Sigma Plot software , with standard concentration on the x-axis and percentage of absorbance on the y-axis. Drew the best-fit curve through the standard points.

Percentage absorbance = **(B –blank OD) / (B_0 –blank OD**) where

B = OD of sample or standard and

Bo = OD of zero standard (total binding)

STANDARD CURVE



Performance Characteristics

Sensitivity

The minimum detectable concentration of Visfatin is 0.778ng/mL

- Detection Range

  0.1-1,000 ng/mL

- Reproducibility

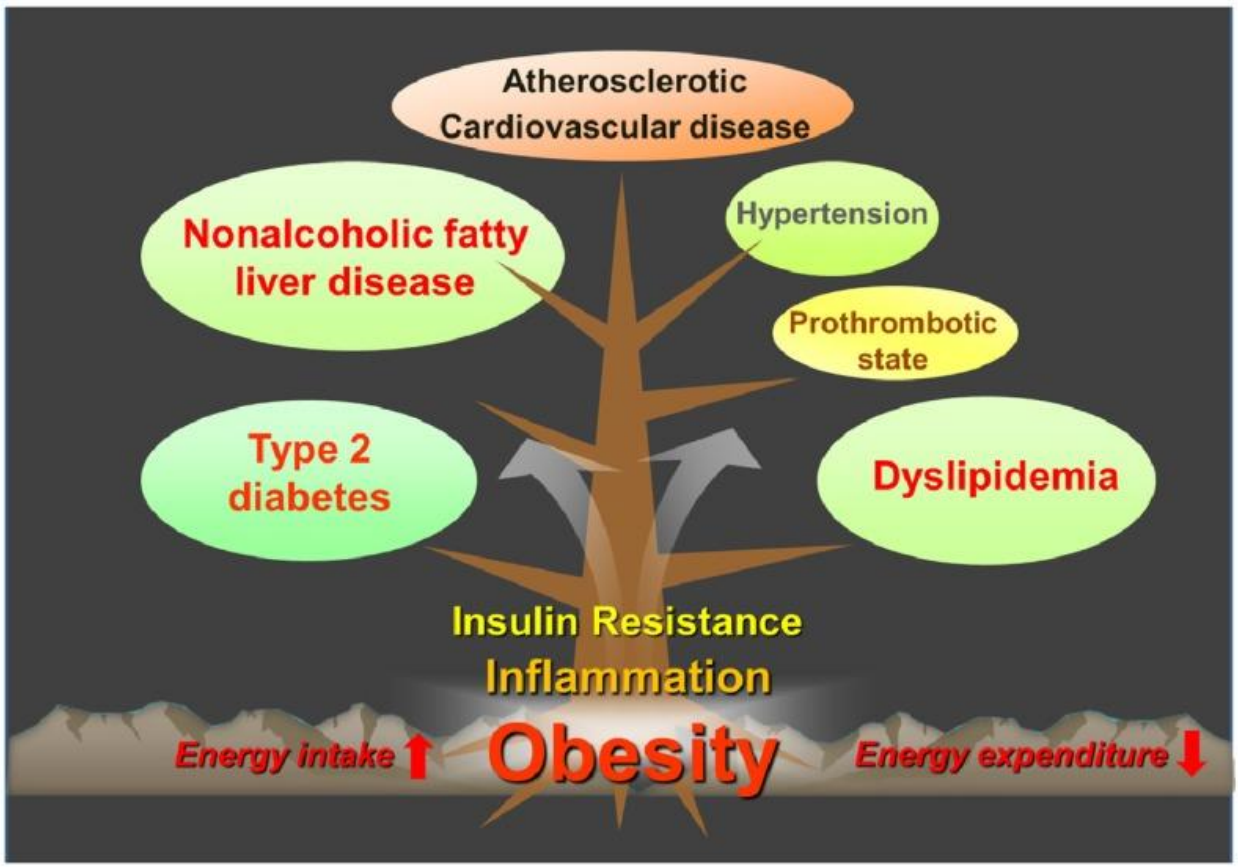
  Intra-Assay: CV<10%

  Inter-Assay: CV<15%

Specificity

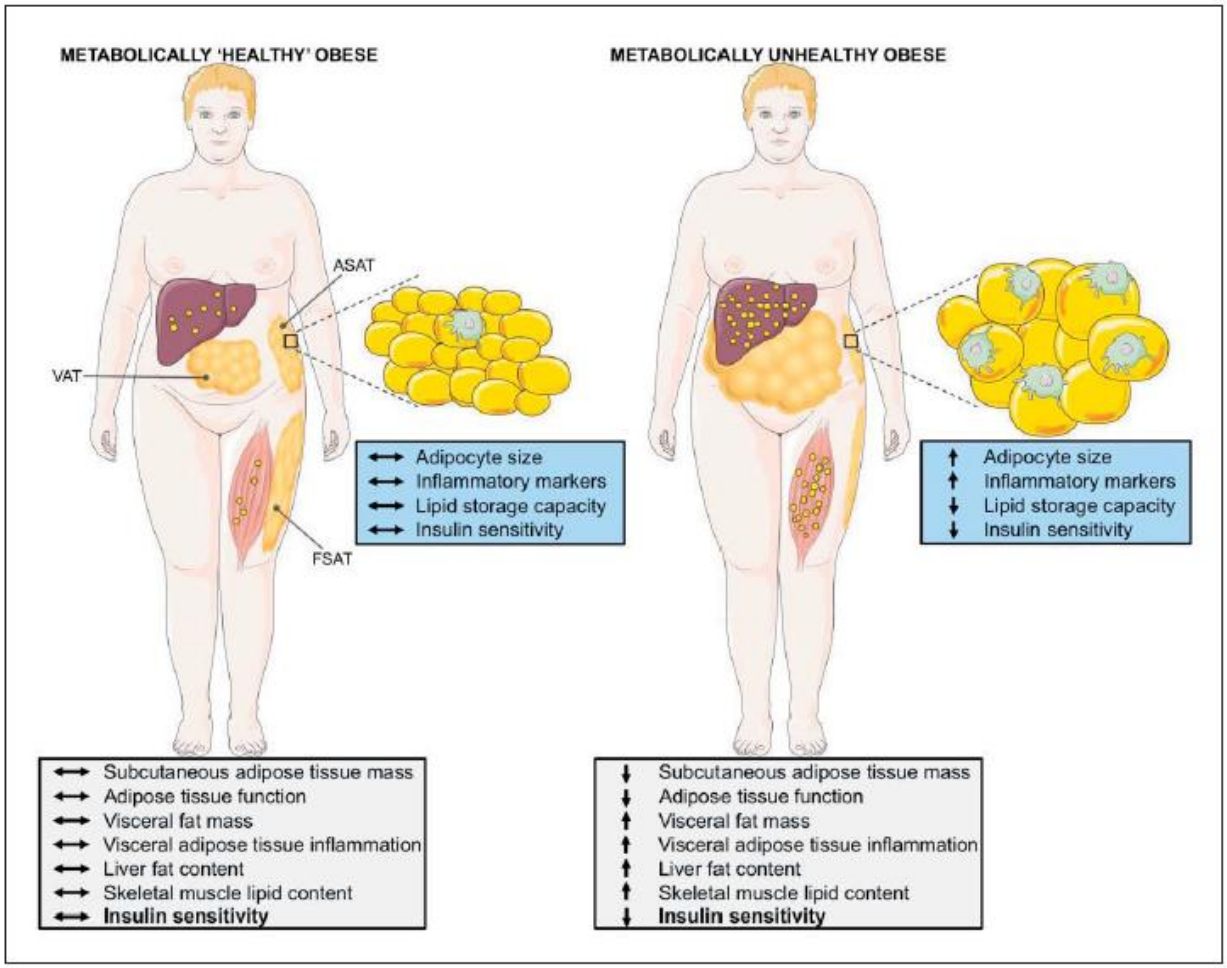
Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested: Ghrelin, Nystatin, NPY and APC.

FIG :1 THE METABOLIC COMPLICATIONS OF OBESITY



Un Ju Jung and Myung-Sook Choi, Obesity and Its Metabolic Complications: The Role of Adipokines and the Relationship between Obesity, Inflammation, Insulin Resistance, Dyslipidemia and Nonalcoholic Fatty Liver Disease Int. J. Mol. Sci.2014, 15(4), 6184-6223;

FIG :2 ADIPOCYTES IN MHO AND MUHO



Gijs H. Goossens The Metabolic Phenotype in Obesity: Fat Mass, Body Fat Distribution, and Adipose Tissue Function Obes Facts 2017;10:207–215.

STATISTICAL ANALYSIS

All the data were entered in the Microsoft excel 2010 and were statistical analysed using SPSS version 20.0.

- Continuous variables in numbers were analysed as mean and standard deviation.

- Descriptive categories were analysed as frequencies and percentages.

- After confirming the normal distribution of continuous variables using normality tests (kolmogrov-smirnov and shapiro -wilk), student T test was done to test the significant difference in means of the study group and the control group.

- For all the statistical tests, significant difference was analysed by P value of <0.05.

- For studying the correlation between variables, Pearson correlation was done.

RESULTS

Table 3 Age Distribution of the study population (n=90)

Age Group	Cases N (%)	Controls N (%)	Total N(%)
19 – 20 yrs	3(5)	1(3.3)	4(4.5)
21 – 30 yrs	12(20)	11(36.6)	23(25.5)
31 – 35 yrs	45(75)	18(60)	63(70)
Total	60(100)	30(100)	90(100)

Mean Age (±SD) : 31.55 (± 3.7)
Minimum Age : 19 yrs
Maximum Age : 35 yrs

Comments:

Distribution of the study population based on age showed about 70% of subjects were in the age group of 31 – 35 yrs and about 25% between 21-30 yrs and 1% in the age group of 19-20 yrs. The mean age of the study population was 31 years.

Fig: 3 Bar chart showing Age distribution of the study population (n=90)

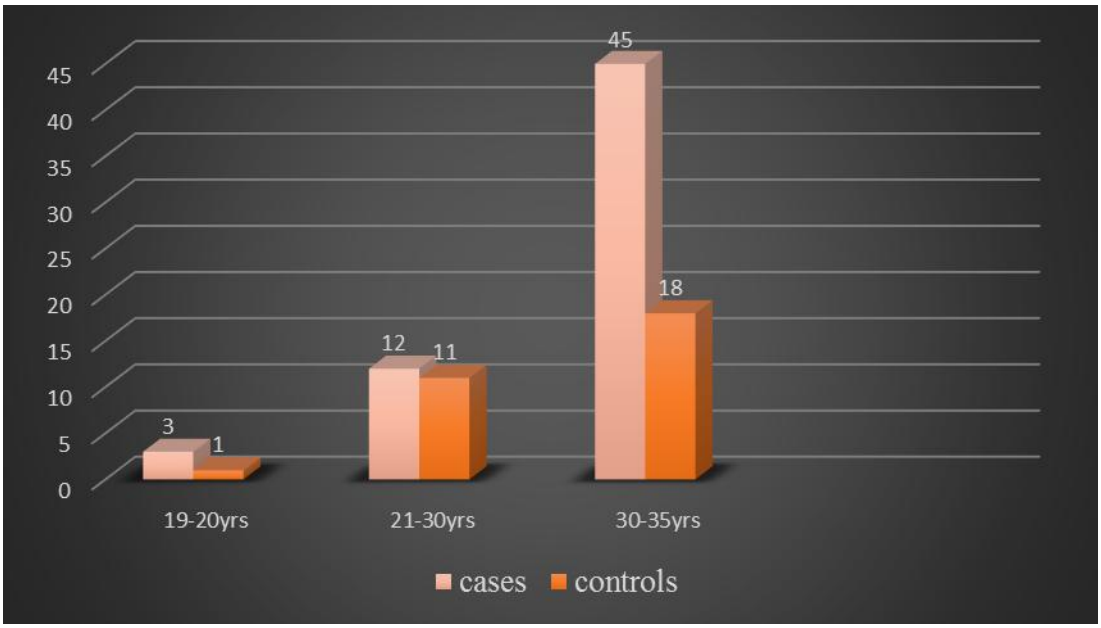


Table 4 Comparision of Age among cases and controls (n=90)

STUDENT T TEST

Group	Mean age	Std deviation	Mean difference	'P' value	95% confidence Interval	
					Lower	Upper
Cases	31.75	3.9	0.47	0.687*		
Controls	31.26	3.5			-0.514	2.881

*** not significant**

Comments:

The mean age difference between the subjects in the cases group and the subjects in the control group was 0.47 and this mean difference was not statistically significant. (P 0.689). Hence both the cases and controls were comparable in terms of age.

Table 5 Gender distribution of the study population (n=90)

Gender	Cases N (%)	Controls N (%)	Total N (%)
Males	**27(45)**	**19(72.8)**	**46(51.1)**
Females	**33(55)**	**11(27.2)**	**44(48.9)**
Total	**60(100)**	**30(100)**	**90(100)**

Comments:

Among the study population of 90 subjects males were 51% and females were 49% with not much difference between both sexes.

Fig:4 Bar chart showing gender distribution of the study population

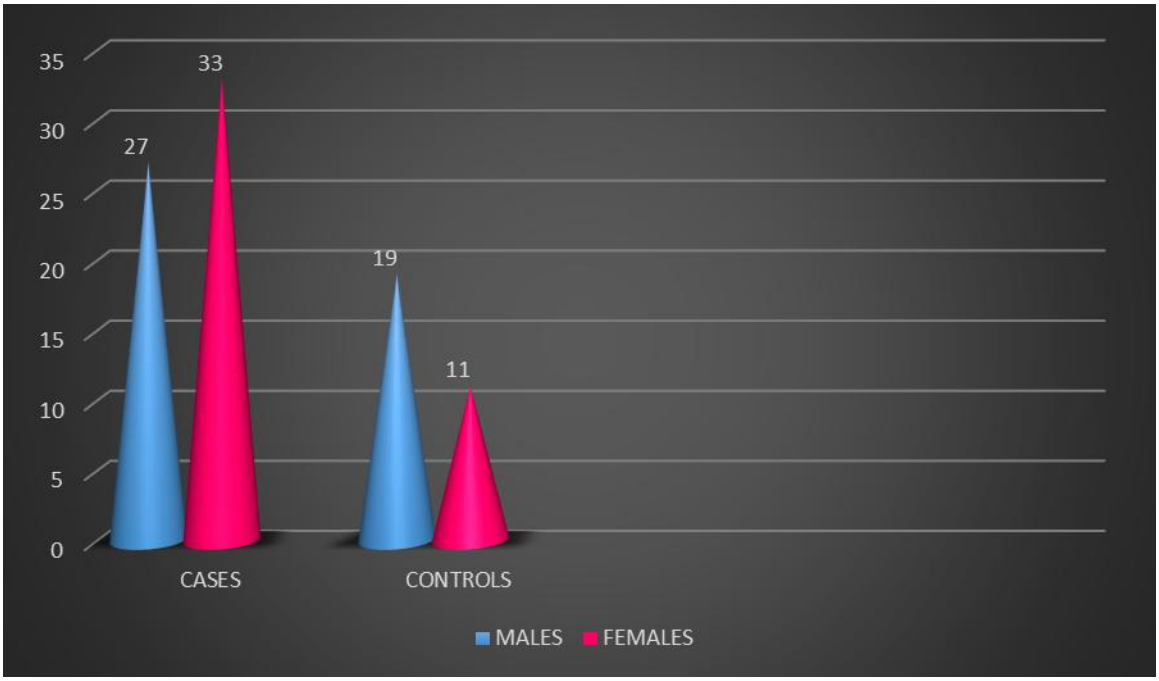
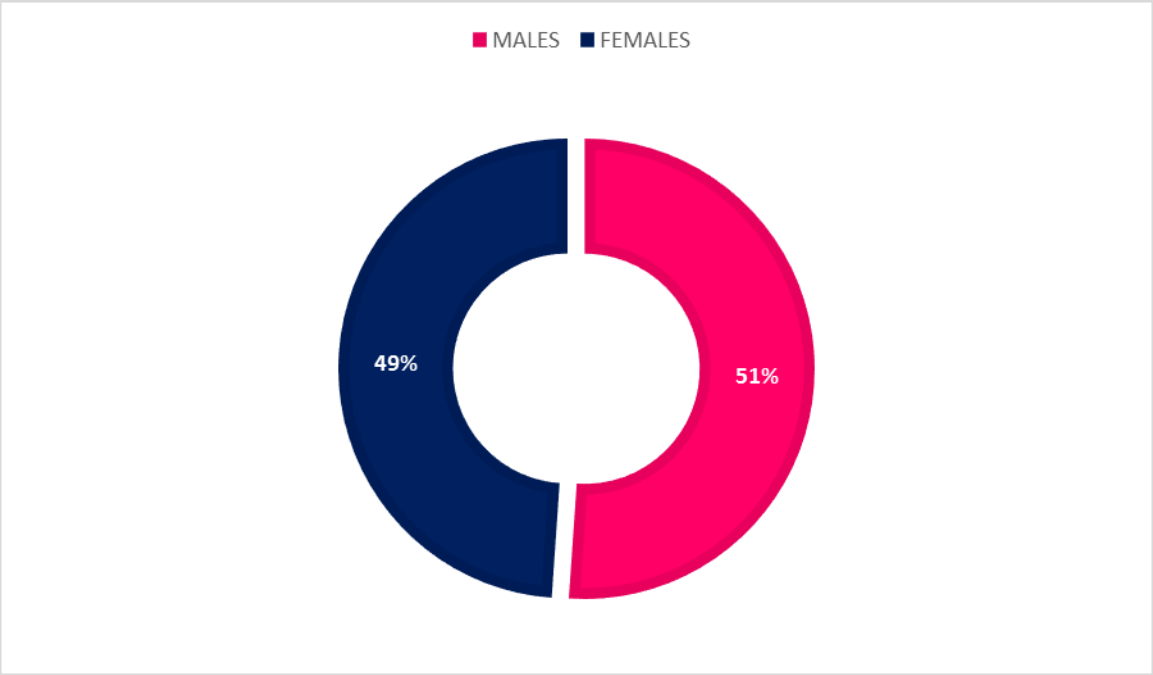


Fig: 5 Doughnut chart showing gender distribution of the study population



Comments

Males and Females were almost equally distributed in both cases and controls P value of 0.139 which is not statistically significant. Hence this study had age-matched and sex matched controls.

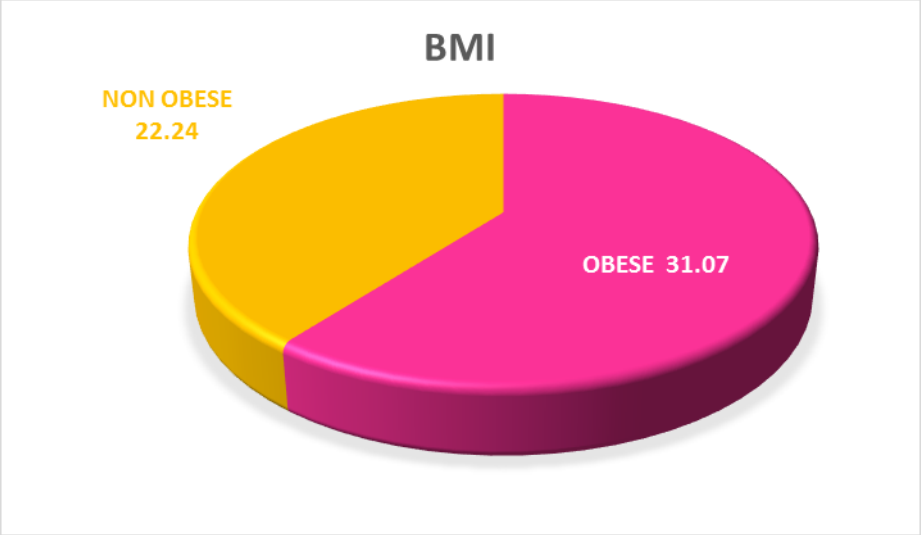
Table: 6 Comparison of BMI among cases and controls (N=90)

Student T test

Group	Mean	Std deviation	Mean difference	P Value	95% confidence Interval	
					Lower	Upper
Cases	31.07	3.70	8.826	0.00	7.40	10.24
Controls	22.24	1.75				

Comments: The BMI among the cases was higher compared to the control with highly significant p value 0.00.

Fig: 5 Pie chart showing distribution of BMI of the study population



Comments: The obese study group had higher mean BMI of 31.07 than the non-obese group of mean BMI 22.24.

Table: 7 Distribution of Anthropometric Indices among the study population

	Group	N	Mean	Mean difference	Student t test p value
Weight kg	**Case**	**60**	**80.00**	**20.92**	**0.01**
	Control	**30**	**59.07**		
Height cm	**Case**	**60**	**160.40**	**-3.20**	**0.011**
	Control	**30**	**163.60**		
BMI	**Case**	**60**	**31.08**	**8.85**	**0.001**
	Control	**30**	**22.22**		
Waist Circumference cm	**Case**	**60**	**99.38**	**24.98**	**0.001**
	Control	**30**	**74.40**		
Hip Circumference cm	**Case**	**60**	**106.97**	**19.43**	**0.01**
	Control	**30**	**87.53**		
Waist-Hip Ratio	**Case**	**60**	**0.927**	**0.082**	**0.02**
	Control	**30**	**0.845**		

Comments:

1. Subjects in the obese study group had higher mean weight (80 kg) than the control group (59 kg) with significant P value of 0.01.

2. The mean height was higher in the control group (163 cm) than the obese group of mean height (160 cm) with significant P value 0.011.

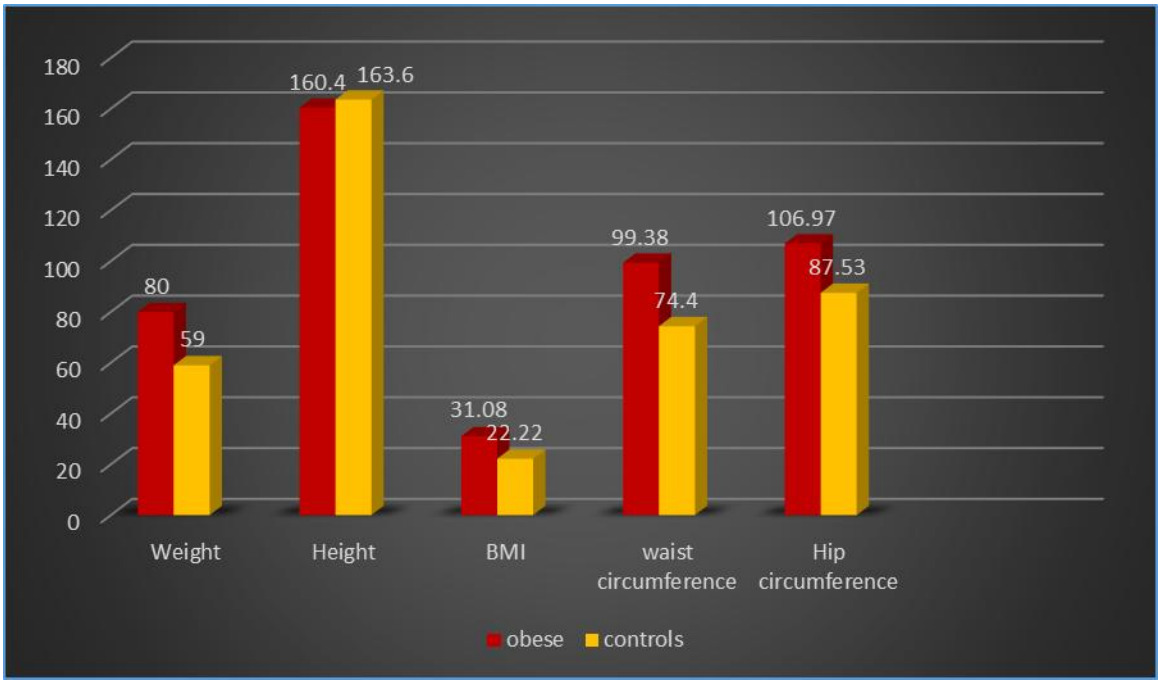
3. Subjects in the obese study group had higher mean BMI (31.08 cm) than the control group (22.22 cm) with significant P value of 0.001.

4. The waist circumference was higher in the obese study group (99.38 cm) than the control group (74.40 cm) with significant P value of 0.001.

5. Subjects in the obese study group had higher mean hip circumference (31.08) than the control group (22.22) with significant P value of 0.01.

6. The waist –hip ratio was higher in the obese study group (0.927) than the control group (0.845) with significant P value of 0.02.

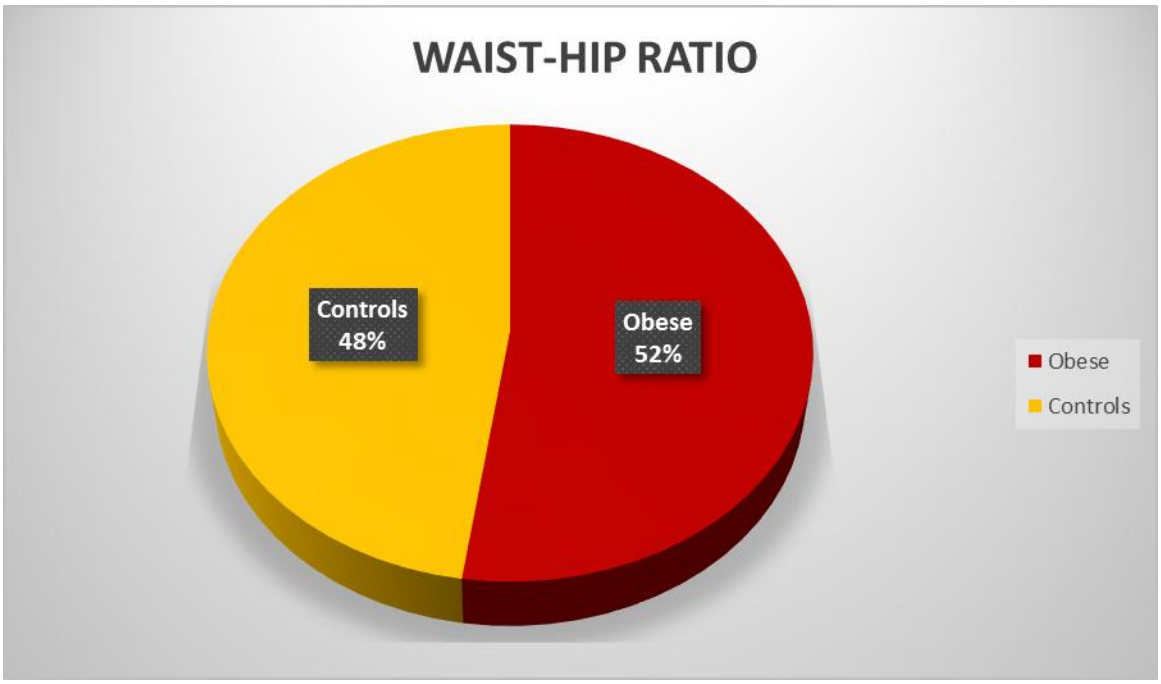
Fig: 6 Bar Chart showing distribution of anthropometric indices among the study population (n=90)



Comments:

The mean of weight, BMI, waist circumference, hip circumference and waist-hip ratio was significantly higher in the obese study group compared to the control group, whereas the mean height was higher in controls than the study group.

Fig: 7 Pie chart showing the distribution of waist – hip ratio among the study population ((n=90)



Comments:

This chart shows percentage of waist – hip ratio higher in the obese Group (51%) than the control group (49%).

Table: 8 Distribution of Lipid profile among the study population

	Group	N	Mean	Mean difference	Student T test p value
Serum T.cholesterol mg/dl	**Case**	**60**	**205.6**	**0.00**	**1.00**
	Control	**30**	**205.6**		
Serum Triglycerides mg/dl	**Case**	**60**	**211.33**	**127.40**	**0.001**
	Control	**30**	**83.93**		
Serum LDL-C mg/dl	**Case**	**60**	**133.83**	**50.70**	**0.00**
	Control	**30**	**83.93**		
Serum VLDL –C mg/dl	**Case**	**60**	**42**	**24.10**	**0.001**
	Control	**30**	**17.9**		
Serum HDL-C mg/dl	**Case**	**60**	**40.20**	**0.93**	**0.52**
	Control	**30**	**41.13**		

Comments:

1. The mean serum total cholesterol levels were equal in both cases and control with insignificant p value 1.00.

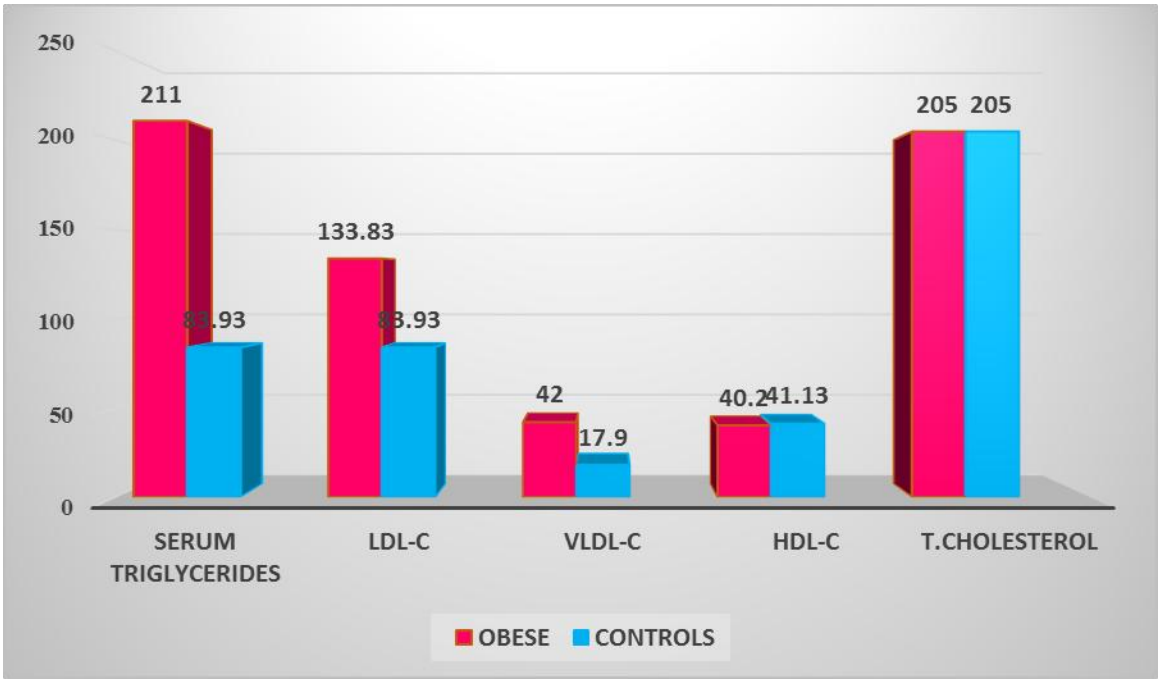
2. Subjects in the obese study group had higher mean serum triglycerides (211mg/dl) than the control group (83 mg/dl) with significant P value of 0.001.

3. The LDL-C was higher in the obese study group (133 mg/dl) than the control group (83 mg/dl) with significant P value of 0.00.

4. Subjects in the obese study group had higher mean VLDL-C (42 mg/dl) than the control group (17 mg/dl) with significant P value of 0.001.

5. The HDL-C was lower in the obese study group (40.20 mg/dl) than the control group (41.13 mg/dl) with insignificant P value of 0.52.

Fig: 8 Bar chart showing distribution of Lipid profile among the study population (N=90)



Comments:

Subjects in the obese study group had higher mean serum triglycerides, LDL-C, and VLDL-C than the control group. The HDL-C was lower in the obese study group than the control group. The serum total cholesterol levels were equal in both the groups.

Table 9 Distribution of Atherogenic index among the study population (N=90)

	Group	N	Mean	Mean difference	Student t test p value
Atherogenic index	**Obese**	**60**	**0.69**	**0.394**	**0.0001**
	control	**30**	**0.30**		

Comments:

Subjects in the obese study group had higher mean atherogenic index (0.69) than the control group (0.30) with significant P value of 0.0001.

Fig: 9 Bar chart showing distribution of Atherogenic index among the study population (N=90)

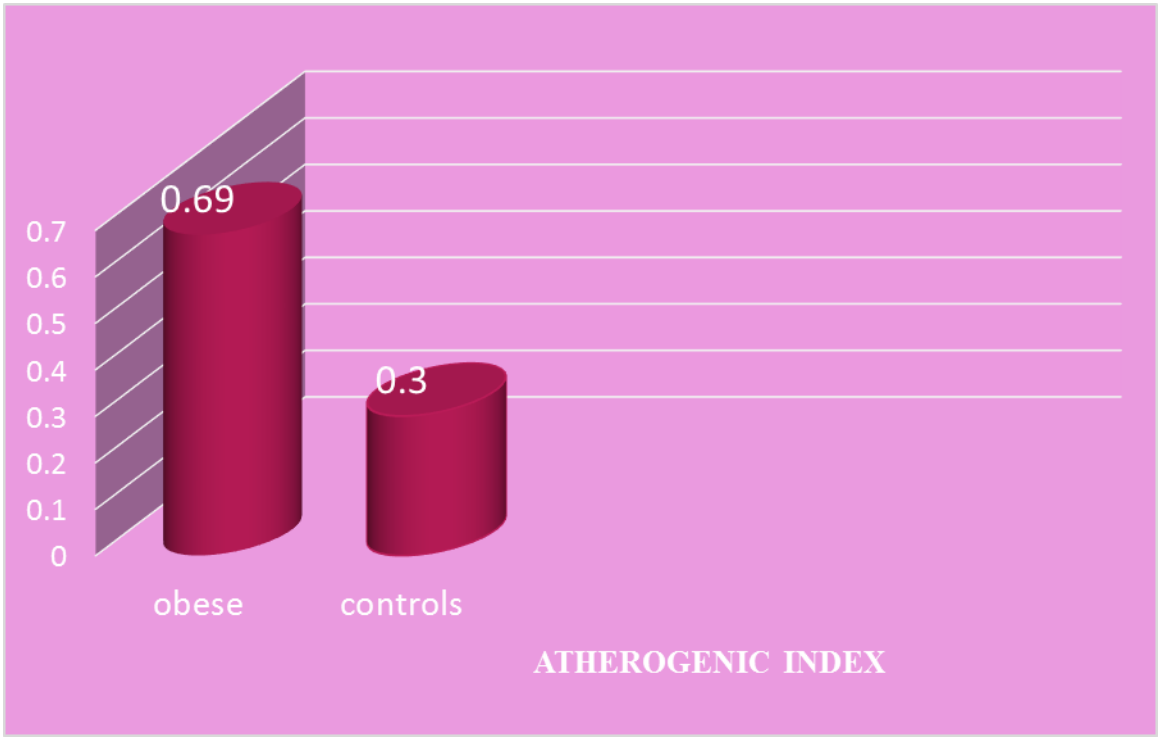


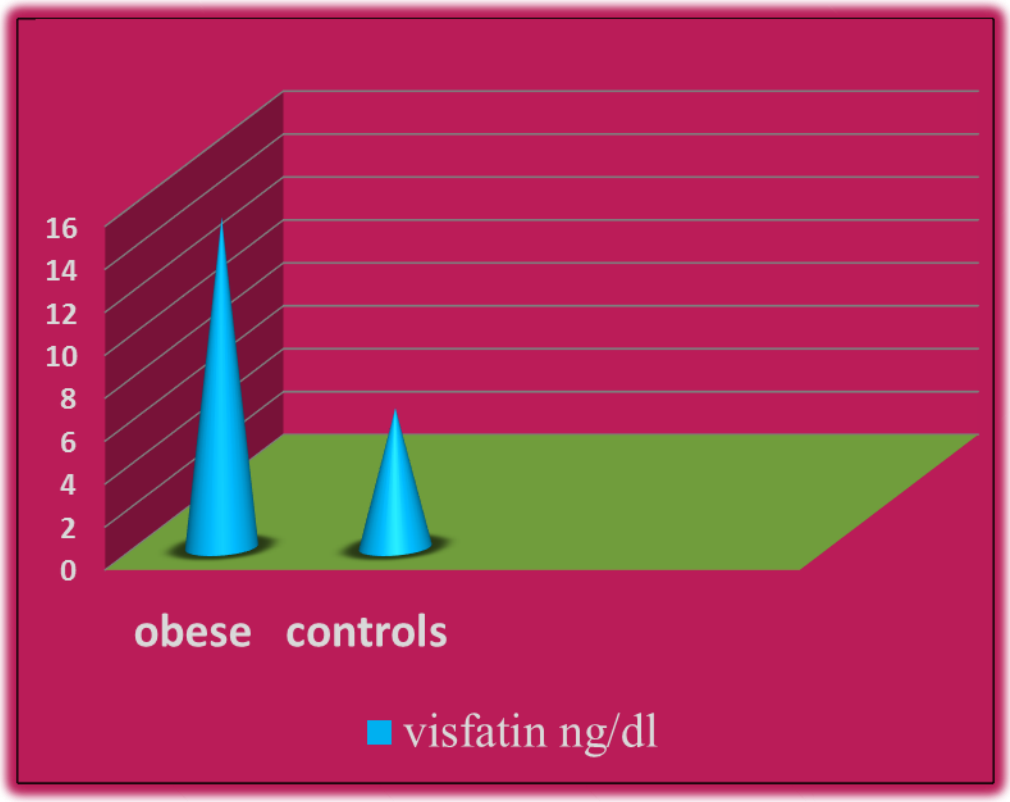
Table: 10 Distribution of Visfatin among the study population

	Group	N	Mean	Mean difference	Student t test p value
Visfatin (ng/dl)	**Obese**	**60**	**15.4**	**8.880**	**0.0009**
	Control	**30**	**6.5**		

Comments:

The mean serum Visfatin levels were higher in the obese study subjects (15.4 ng/dl) than the control group (6.5 ng/dl) with significant p value of 0.00.

Fig: 10 Pie chart representation of distribution of Visfatin among the study population (N=90)



CORRELATION STUDIES

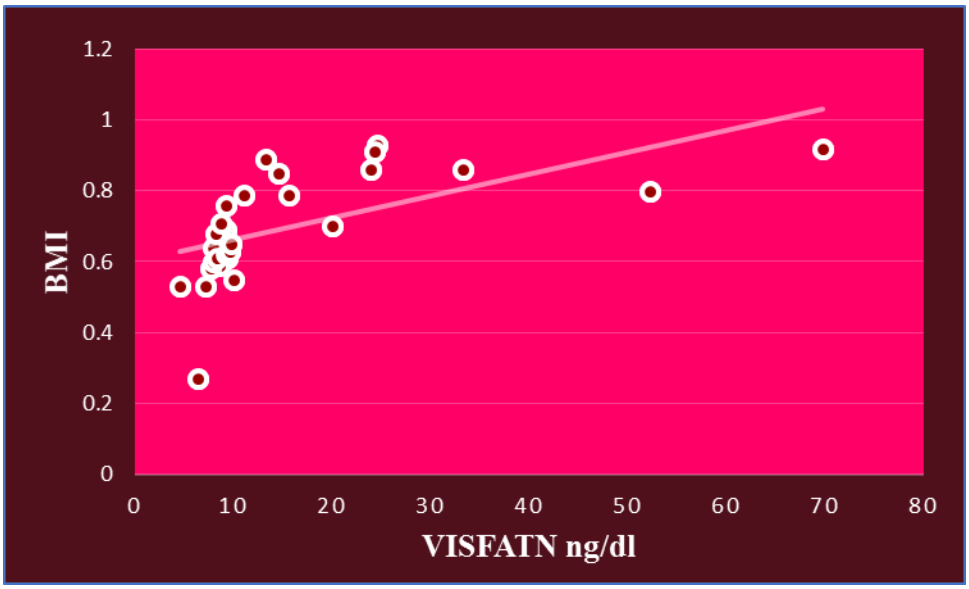
Table 11 Correlation between Visfatin and BMI among obese young adults

	r value	P value
Pearson correlation	**0.927**	**0.00**

Comments:

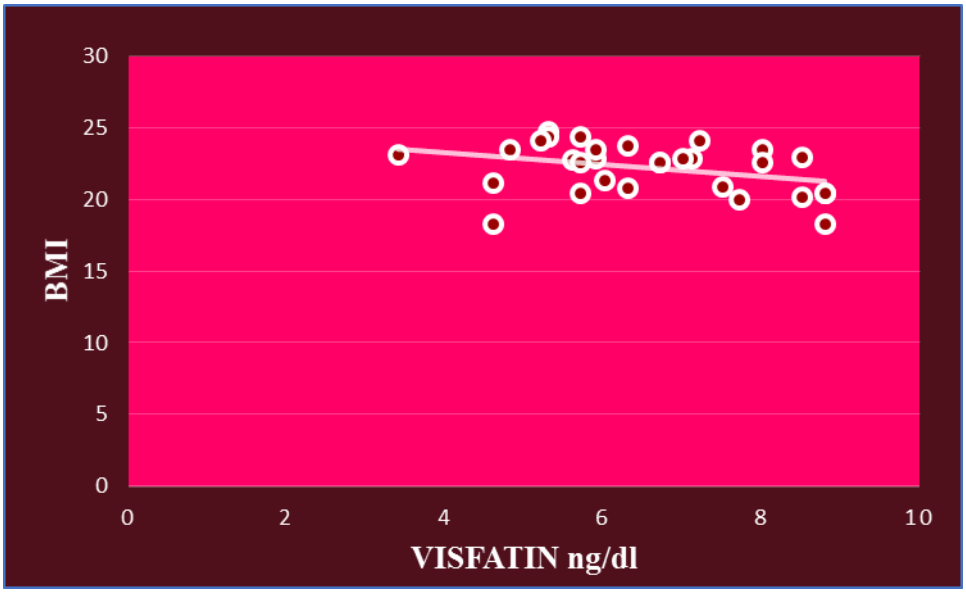
The association between Serum Visfatin and BMI shows positive correlation, pearson correlation constant r = 0.927 (P: 0.00).

Fig: 11 Scatter plot showing Correlation between Visfatin and BMI obese group



Comments: This chart shows linear positive correlation between BMI and serum visfatin among the obese study group.

Fig: 12 Scatter plot showing Correlation between Visfatin and BMI in Control group



Comments:

Serum visfatin levels and BMI showed negative correlation among the control group.

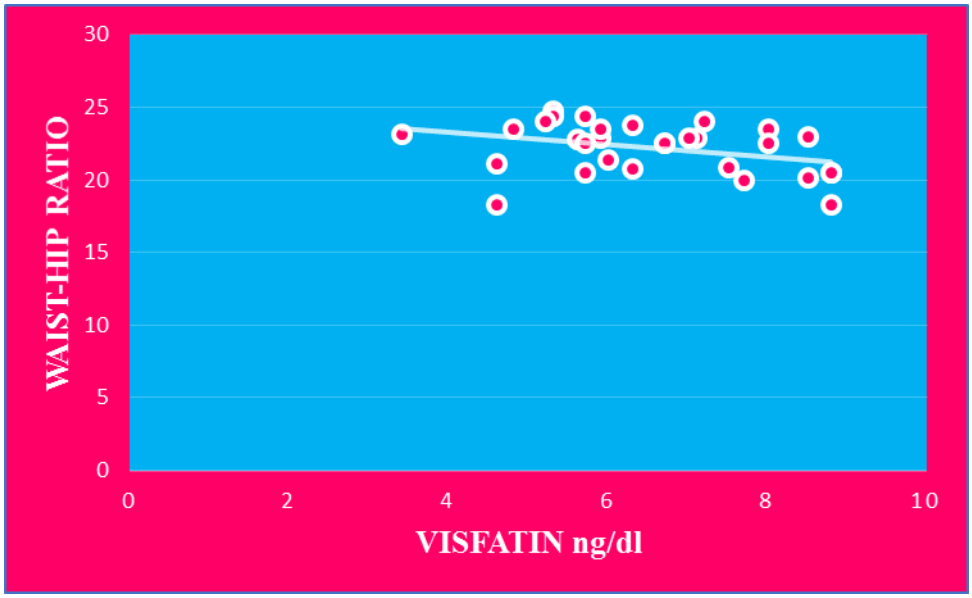
Table 12 Correlation between Visfatin and Anthropometric Indices among obese young adults

	Pearson correlation r value	p value
WAIST CIRCUMFERENCE (cm)	**0.725**	**0.00**
HIP CIRCUMFERENCE (cm)	**0.106**	**0.419**
WAIST/HIP RATIO	**0.659**	**0.00**

Comments:

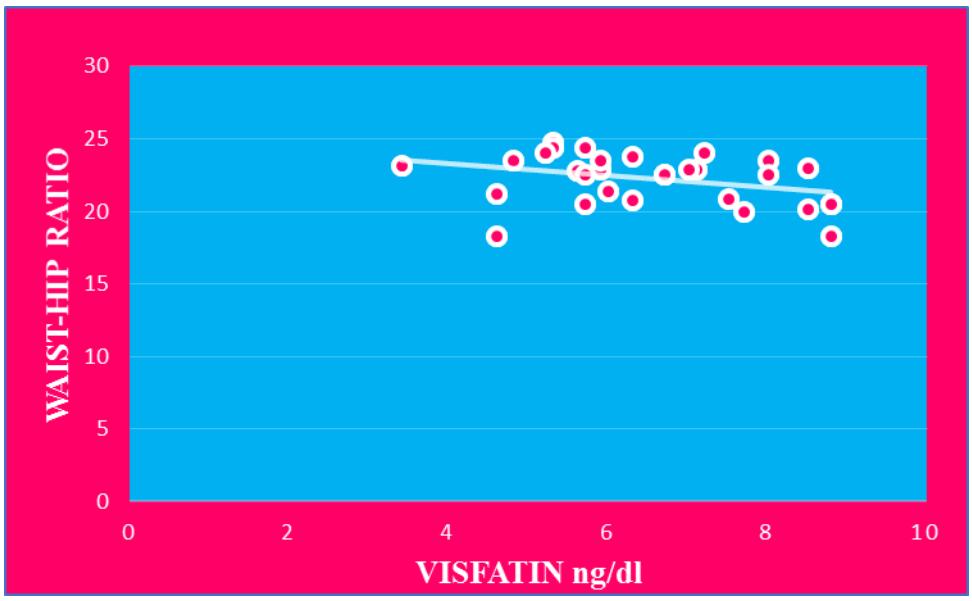
1. There was positive correlation between Serum Visfatin and waist circumference and Waist – Hip Ratio (P 0.00).
2. There was positive correlation between Visfatin and Hip circumference but with insignificant P value 0.419

Fig: 13 Scatter plot showing Correlation between Visfatin and Waist-Hip Ratio (WHR) in Obese group



Comments: There was positive correlation between Atherogenic index and serum visfatin among the obese study group

Fig:14 Scatter plot showing Correlation between Visfatin and Waist-Hip Ratio (WHR) in Control group



Comments: Serum visfatin levels and serum triglycerides showed negative correlation among the control group.

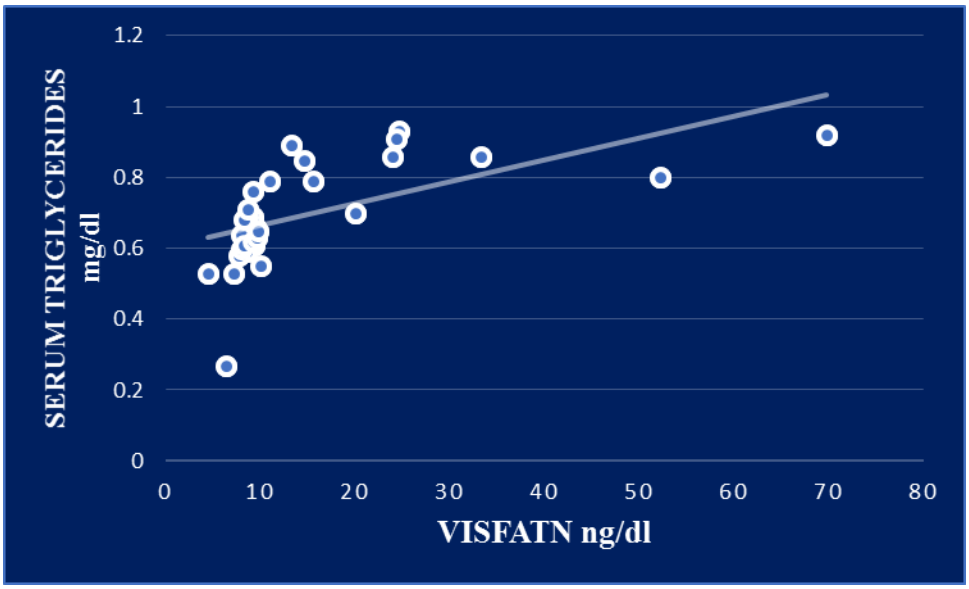
Table 13 Correlation between Visfatin and Lipid Profile among obese young adults

	Pearson correlation r value	**p value**
Serum T.cholesterol mg/dl	**0.010** **(p:0.938)**	**0.938**
Serum Triglycerides mg/dl	**0.32**	**0.04**
Serum LDL-C mg/dl	**0.911**	**0.00**
Serum VLDL –C **mg/dl**	**0.355**	**0.05**
Serum HDL-C mg/dl	**-0.725**	**0.00**

Comments:

1. There is positive correlation between Visfatin and serum triglycerides, LDL-C, VLDL –C, and Total Cholesterol. p value being significant for only serum triglycerides, LDL-C, VLDL –C ($P \leq 0.05$) and insignificant for Total Cholesterol ($p > 0.938$)
2. There is negative correlation between Visfatin and HDL-C.

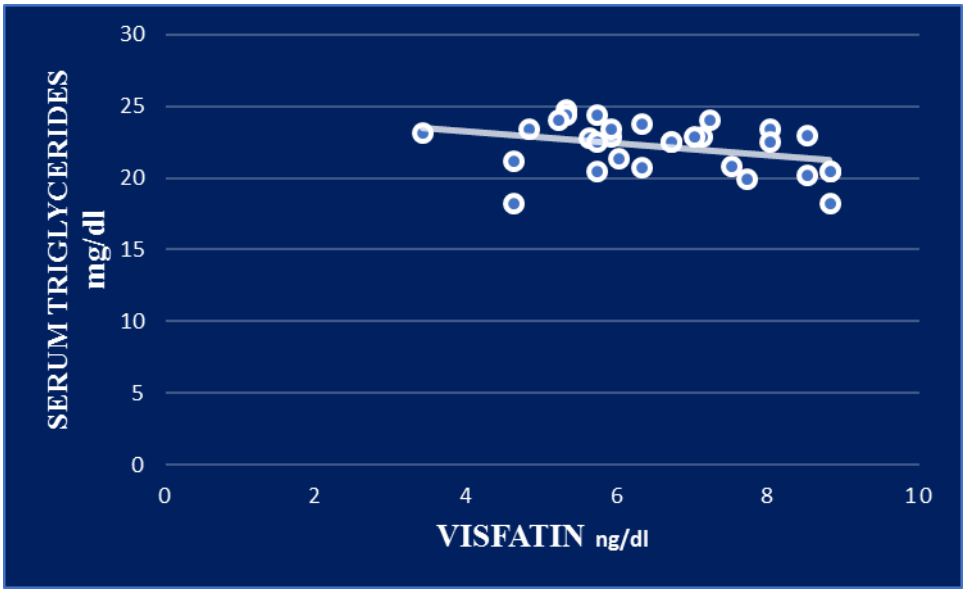
Fig: 15 Scatter plot showing Correlation between Visfatin and Serum Triglycerides in obese group



Comments:

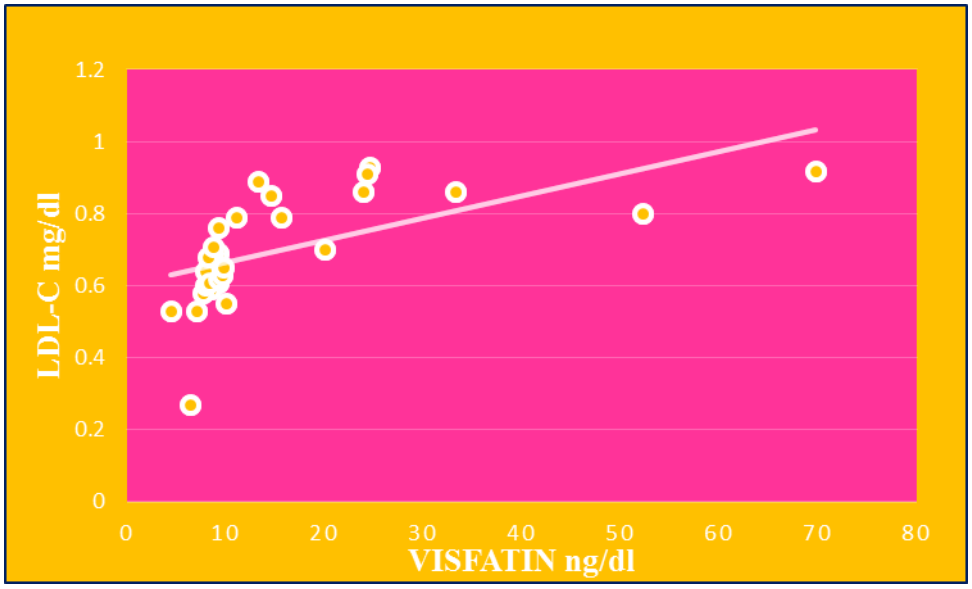
Among the obese study group, there was positive correlation between serum visfatin levels and serum triglycerides.

Fig:16 Scatter plot showing Correlation between Visfatin and Serum Triglycerides in Control group



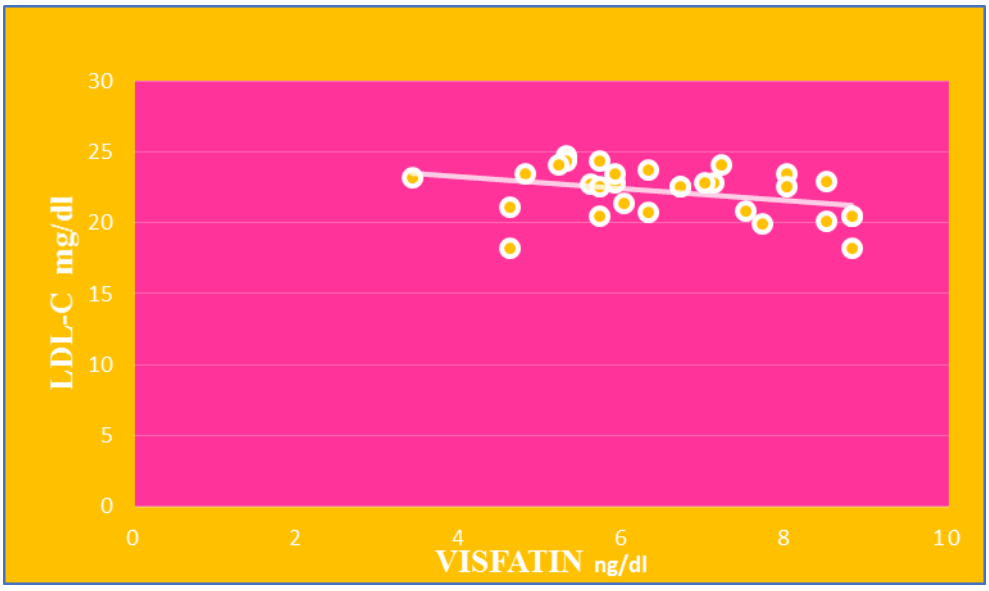
Comments: Serum visfatin levels and serum triglycerides showed negative correlation among the control group

Fig:17 Scatter plot showing Correlation between Visfatin and LDL-C in obese group



Comments: There was positive correlation between LDL-C and serum visfatin among the obese study group

Fig: 18 Scatter plot showing Correlation between Visfatin and LDL-C in control group



Comments:

Serum visfatin levels and LDL-C showed negative correlation among the control group.

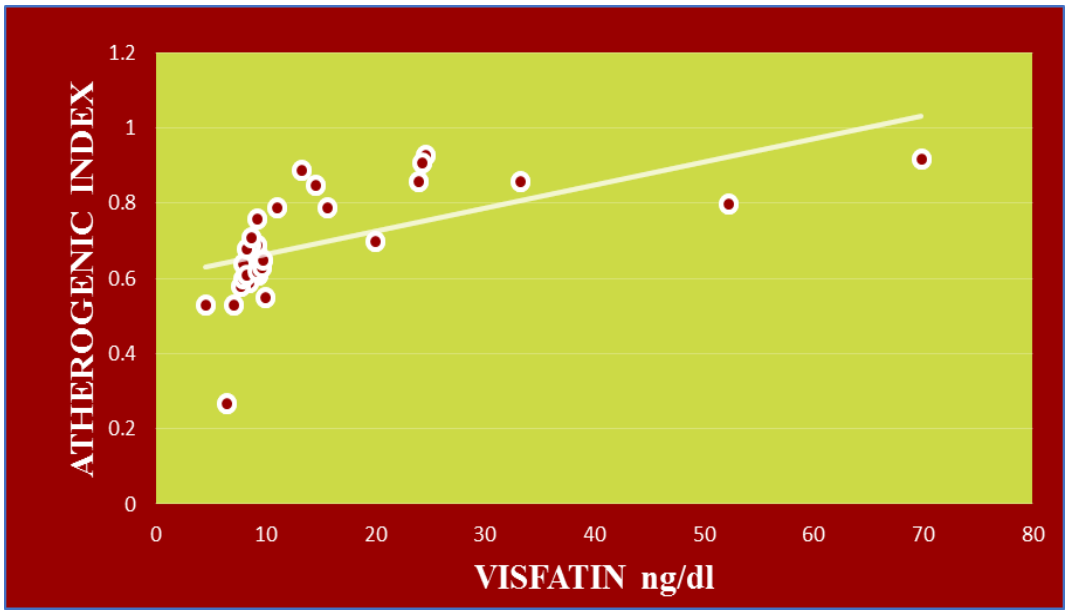
Table 14 Correlation between Visfatin and Atherogenic index among obese young adults

	r value	P value
Pearson correlation	**0.599**	**0.00**

Comments:

The association between Serum Visfatin and Atherogenic index shows positive correlation, Pearson r = 0.599 (P: 0.00).

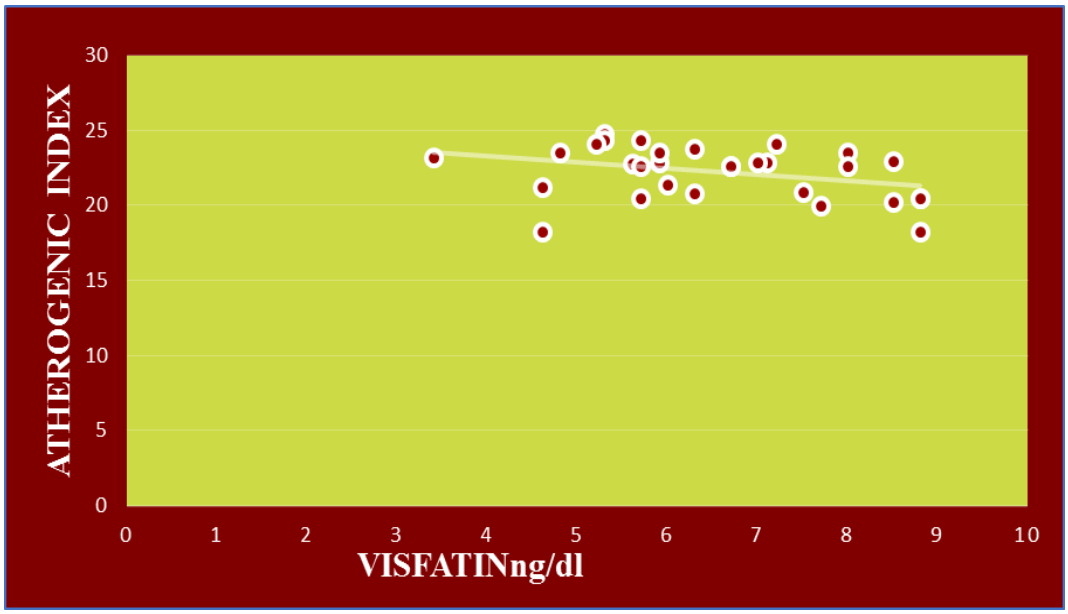
Fig:19 Scatter plot showing Correlation between Visfatin and Atherogenic index in obese group



Comments:

The chart shows linear positive correlation between Atherogenic index and serum visfatin among the obese study group.

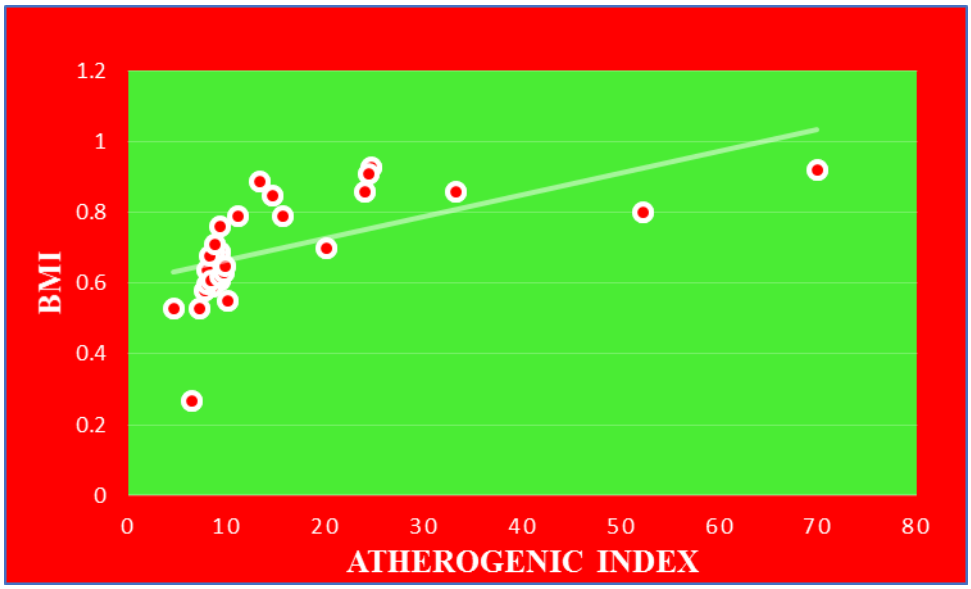
Fig: 20 Scatter plot showing Correlation between Visfatin and Atherogenic index in Control group



Comments:

Atherogenic index and serum visfatin levels showed negative correlation among the control group.

Fig: 21 Scatter plot showing Correlation between BMI and Atherogenic index in obese group



Comments:

This graph shows positive correlation between Atherogenic index between BMI among the obese study group with r value : 0.568 (P 0.01).

DISCUSSION

This study had been designed to evaluate the serum Visfatin levels in young obese adults (60) and compared with non-obese individuals, all the subjects matching the inclusion and exclusion criteria. All the 90 subjects were young adults in the age group of 19-35 years. The mean age of cases and controls were 31.75 and 31.26 respectively, with mean difference of 0.47 ($P < 0.687$). This shows that there is no significant difference between the two groups in terms of age and they were age-matched.

Both males and females were included in this study. About 51% were males and 49% were females with no significant difference ($P < 0.139$) and were sex – matched. The serum levels of new adipokine, Visfatin was estimated in both cases and controls.

Fukuhara et al in 2005 showed that Visfatin – 55 kDa protein is a newly identified adipokine, expressed preferentially in visceral adipose tissue (VAT) than subcutaneous adipose tissue (SAT) in humans. He demonstrated that in KKAy obese mice, fed with high fat diet ,the plasma levels of this adipokine increases with the development of obesity[21].

Haider et al found that the plasma visfatin levels were increased in obese children of age 11-16 years compared to non-obese age-matched control children[81]. Derya Taskeen et al in 2012[31] and Kaminska A[82]et al in 2010 demonstrated the same positive correlation between plasma visfatin levels and adiposity in adolescents and adults respectively.

In this study, serum levels of Visfatin in obese subjects were higher in young obese adults than age-matched non-obese controls which is consistent with the previous studies. The mean visfatin levels among the obese group was 15.4 and of the

control group was 6.5 with significant P value of 0.00. Serum visfatin levels, in this study did not show any sex-related differences, which was also consistent with the results of Fukuhara et al[21] and Haider et al[1].

In this study regarding the anthropometric indices and body fat distribution, the BMI was found to be higher in the obese group than the non obese group, with mean ±SD being 31.08 ± 3.6 in obese group and 22.22 ± 1.7 in non-obese group (P 0.00).The Waist Circumference (WC) and the Hip Circumference (HC) were also higher among the obese subjects than the control group with significant P value of 0.00. The Waist-Hip Ratio (WHR) was significantly higher in the obese groups the mean ± SD, 0.927 ± 0.098 than in the non- obese controls whose mean ±SD was 0.845± 0.039 with P value of 0.001.

The study group, the young obese adults have higher serum triglycerides and LDL-C, than the non-obese control groups with significant difference p value of 0.00. There was not significant difference in serum total cholesterol levels between cases and controls in this study. The obese study group also had higher levels of serum high HDL-C than the control groups.

Urbina EM et al [86] in 2013, Hassan M. Salama et al [4] in 2015 and several other studies had positive correlation between obesity and TG, LDL-C and negative correlation with HDL-C.

Among the obese study group, the correlation between visfatin levels and each anthropometric indices (BMI,WC,HC,WHR) were analysed individually using Pearson correlation tests and there was significant positive correlation between serum visfatin levels and BMI (correlation coefficient r value: 0.927 $P < 0.00$). Serum Visfatin and Waist Circumference had a significant positive correlation

(r 0.725 ; P < 0.00). Serum Visfatin and Hip Circumference also showed positive correlation with insignificant P value of 0.419. The correlation between Waist-Hip Ratio and serum Visfatin levels was significantly positive, with r 0.659 and P value 0.00.

Davutoglu et al [83], in his study also found a positive correlation between plasma Visfatin levels and BMI, waist circumference and waist-hip ratio.Berndt et al[84] in 2005, reported that plasma Visfatin levels was positively correlated with BMI, WC and WHR in his study. Pageno et al [85] in 2006, found a negative correlation of serum visfatin with BMI and WHR in individuals of wider range of obesity. Derya Taskeen et al[31] in 2012, had negative correlation between serum visfatin levels and body fat, BMI and waist-hip ratio.

This difference in the correlation between plasma visfatin levels and the anthropometric indices and body fat distribution may be attributed that visfatin must have different sources in the body other than the adipose tissue and further explorative studies regarding the role and sources of visfatin are needed for further clarification. These variable results also suggest that the circulating visfatin is related to adiposity only if there is remarkable differences in the adiposity between the study groups and the control groups.

Moreover, in this study there was a positive correlation (P< 0.04) between serum visfatin and serum triglycerides (TG) with r value 0.32. The correlation between serum visfatin and low density lipo-protein-cholesterol (LDL-C) was also positive with r 0.911 (P< 0.00). There was negative correlation of serum visfatin with serum HDL-C with r:-0.725 (P < 0.00).

María Teresa Martínez Larrad et al[24] in their study in 2016, also had a positive correlation between circulating visfatin levels and triglycerides and low density

lipoprotein- cholesterol and a negative correlation with high density lipoprotein-cholesterol. Similar positive correlations was observed by Romacho T et al [87] in 2009.

Vanhoutte PM[88] in 2009, when evaluating for the endothelial dysfunction of coronary arteries in obese individuals found the association of altered lipid profile in obese patients. Takebayashi K et al [89] in 2007 also found dyslipidemia was associated with obese type 2 diabetes mellitus patients.

This association between circulating visfatin levels and dyslipidaemia indicates that visfatin, an insulin-mimetic protein have profound effects on lipid homeostasis and triglyceride metabolism.

Atherogenic index of plasma is calculated as logarithmically transformed ratio of serum triglyceride to high density lipoprotein cholesterol (HDL-C). Altered lipid profile in obesity had been in close association with atherosclerosis. Several studies have demonstrated the positive correlation between obesity and atherosclerosis.

Ye Chang et al[10] in 2016, demonstrated the atherogenic index of plasma calculated by log (TG/HDL) as the best and strong predictor of atherosclerosis and cardiovascular risks. The strong correlation between atherogenic index of plasma and the lipoprotein particle size explains the high predictive value of AIP in cardiovascular risk and Dobiasova M [90] in 2006 pointed AIP to be the significant predictor of cardiovascular risk for both research and for practice. AIP which is a sensitive marker of altered lipid profile can be easily calculated from the standard values of lipid profile.

In this study, the obese study population had a significantly higher atherogenic index than the non-obese control group with P value 0.00. Serum visfatin levels showed a positive correlation with atherogenic index with pearson correlation

constant r value to be 0.599 (P<0.00). Atherogenic index also had a positive correlation with BMI of the obese study group (r value : 0.568 p:0.01).

Hanan Mahmoud Fayed et al[91] in 2017 showed a positive correlation between plasma visfatin levels and atherogenic index and dyslipidaemia in patients with end stage renal disease. G A Martos – Moreno et al[92] in 2011 also found a positive correlation between atherogenic index and plasma visfatin levels in obese prepubertal children. Abir Naguib et al[93] showed that plasma visfatin and atherogenic index were negatively correlated in rheumatoid arthritic patients. Ondrej-glunda et al[94] in 2014 also found a negative correlation between plasma visfatin levels and atherogenic index and dyslipidemia in rheumatoid arthritis patients.

Visfatin, also known as nicotinamide phosphoribosyl transferase (Nampt) plays a vital role in regulating cell energy balance, modulating glucose and lipid metabolism. Visfatin though predominantly secreted from the visceral white adipose tissue is also produced by variety of cells including lymphocytes, monocytes, neutrophils and hepatocytes and reported to be associated with several markers of inflammation and endothelial dysfunction[95].

Endothelial dysfunction being the early step in the pathogenesis of atherosclerosis, visfatin is known to enhance the production of various proinflammatory cytokines, activation of leucocytes and synthesis of adhesion molecules that together accounts for the formation of atherosclerotic plaque[96].

The results of this study indicate that serum visfatin levels were higher in obese young adults and visfatin had a positive correlation with anthropometric indices (BMI, WC,HC and WHR), serum triglycerides, LDL-C and atherogenic index. Further longitudinal studies with large sample size are needed to corroborate and confirm these results.

CONCLUSION

Obesity is the most serious risk factor for chronic diseases and its metabolic complications that accounts for high morbidity and mortality rate. Visceral adipose tissue is more metabolically active than subcutaneous adipose tissue and is of great importance for the development of obesity related complications. The adipokine, Visfatin mainly produced and secreted by the visceral adipocytes is enhanced in obese individuals with more visceral fat accumulation.

In this study, the body mass index, the waist circumference and the waist-hip ratio have shown a positive correlation with serum visfatin proving the fact that visfatin is greatly expressed in obese individuals with abdominal or visceral fat accumulation. This clearly indicates that body fat distribution (subcutaneous or visceral) is an important marker for assessing the metabolic risks of an obese individual.

Serum visfatin, having a positive correlation with atherogenic index demonstrates that the metabolically active visceral fat accounts for dyslipidemia and high levels of serum triglycerides and LDL-C which initiates atherosclerotic phenomena in young obese individuals. This indicate that the visceral fat accumulation leads an obese individual into various comorbid conditions including initiation of atherosclerosis even in young adults.

Regarding the role of visfatin as an insulin-mimetic protein, in glucose metabolism conflicting results from various studies about the serum levels of visfatin in diabetes patients suggest, yet more research are needed to elucidate the exact function and source of visfatin. From this study, it is suggested that plasma/serum visfatin can be considered as a potential biomarker for the amount of visceral fat in an obese individual and its impact on atherosclerosis and other metabolic complications that ensues.

LIMITATIONS

The limitations of this study include

- Small sample size of study population
- Single - centered study
- Assessment of body fat distribution by simple methods such as waist circumference (WC), waist-hip ratio (WHR) instead of more advanced accurate methods like MRI/CT.

Additional prospective multi-centric studies, with large sample size of obese individuals and measurements of body fat with high tech methods, such as MRI/CT are warranted to corroborate the results of this study.

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CASE PROFORMA

Name: I.P/O.P No:

Age: Sex:

Address:

Occupation:

Diet: Veg /Non veg

Present complaints:

Past history (History of diabetes/ Hypertension/ thyroid disorders):

Personal history:

Marital history:

Obstetric History (Pregnant history/under OCP pills):

General Examination:

B.P: Pulse:

RS:

CVS:

Abd:

CNS:

ANTHROPOMETRIC INDICES:

PARAMETERS

Height in meters :

Weight in Kg :

BMI :

WC in cm :

HC in cm :

Waist hip ratio:

INVESTIGATIONS :

Plasma Visfatin ng/mL :

TC mg/dL :

TG mg/dL :

LDL-C mg/dL :

HDL-C mg/dL :

VLDL-C mg/dL :

Atherogenic Index (logTG/HDL-C) :

Fasting Glucose mg/dl :

MASTER CHART - CASES

S.NO	AGE	SEX	Sys .BPmmHg	Dias.BP mmHg	Height cm	Weight kg	BMI	Waist circumference cm	Hip circumference cm	Waist / Hip Ratio	T.CHO mg/dl	TRIGLY mg/dl	LDL mg/dl	HDL mg/dl	VLDL mg/dl	FBS mg/dl	Atherogenic Index	Plasma Visfatin ng/ml
1	34	1	126	82	159	73	28.9	92	97	0.94	223	164	99	48	33	90	0.53	7.1
2	20	2	130	70	155	70	29.1	94	100	0.94	146	210	108	43	42	90	0.68	8.2
3	35	2	126	70	164	90	33.9	110	100	1.1	99	258	151	35	51	80	0.86	23.9
4	34	2	126	80	162	75	28.6	95	103	0.92	206	176	117	42	35	86	0.62	9.2
5	35	2	110	70	158	92	36.2	126	118	1.06	284	240	146	33	48	90	0.86	33.2
6	35	2	126	82	159	76	30.2	96	102	0.94	173	175	120	41	35	82	0.63	9.6
7	32	2	126	80	160	110	43.7	136	108	1.25	187	234	261	28	46	88	0.92	69.8
8	34	2	118	70	165	80	29.8	95	102	0.93	224	191	132	42	38	86	0.65	9.7
9	32	2	120	78	156	80	32.4	116	102	1.13	202	176	182	35	35	91	0.7	20
10	33	2	118	78	160	102	39.5	128	108	1.18	173	210	224	33	42	86	0.8	52.2
11	33	2	108	70	154	72	30.8	105	98	1.02	186	144	138	40	28	84	0.55	10
12	35	1	126	80	161	74	28.9	87	96	0.9	170	240	119	46	48	95	0.71	8.7
13	34	1	120	76	162	86	32	92	98	0.98	223	257	149	36	51	88	0.85	14.6
14	30	2	116	76	158	69	27.9	98	108	0.8	171	165	87	56	33	87	0.27	6.4
15	34	1	116	78	164	76	28.4	98	118	0.92	199	178	126	43	35	97	0.61	9.3
16	25	1	100	70	164	96	35.6	113	105	1.1	197	310	160	36	62	70	0.93	24.6
17	32	1	116	72	176	91	29.2	115	107	1.14	210	289	159	35	57	85	0.91	24.3
18	34	1	110	72	158	70	28.2	99	111	0.89	143	182	109	46	36	83	0.59	8.5
19	35	1	120	80	161	89	34.1	108	94	1	215	213	145	34	42	98	0.79	15.6
20	30	2	114	70	151	67	29	97	115	0.82	185	181	93	47	36	83	0.58	7.7

MASTER CHART - CASES

S.NO	AGE	SEX	Sys .BP mmHg	Dias.BP mmHg	Height cm	Weight kg	BMI	Waist circumference cm	Hip circumference cm	Waist / Hip Ratio	T.CHO mg/dl	TRIGLY mg/dl	LDL mg/dl	HDL mg/dl	VLDL mg/dl	FBS mg/dl	Atherogenic Index	Plasma Visfatin ng/ml
21	31	2	118	70	158	75	30.5	99	112	0.88	284	210	132	45	42	82	0.66	9.4
22	34	2	120	70	158	74	29.8	97	115	0.82	230	190	100	47	38	72	0.6	7.9
23	35	1	100	68	163	80	30.2	110	116	0.94	164	205	128	41	41	84	0.69	9.2
24	34	1	110	70	165	88	32.2	108	112	0.96	157	285	144	36	57	78	0.89	13.3
25	32	1	100	70	168	81	28.4	88	102	0.8	187	213	98	48	42	86	0.64	7.9
26	31	1	112	78	172	82	27.9	89	114	0.85	230	219	118	44	43	93	0.69	8.7
27	34	1	100	72	156	79	32.5	92	97	0.95	197	226	144	36	45	92	0.79	11
28	19	2	98	60	152	66	28.5	96	110	0.84	203	187	119	45	37	70	0.61	8.3
29	31	2	100	68	159	73	28.9	97	112	0.95	198	231	128	40	46	98	0.76	9.2
30	28	2	96	60	154	64	26.9	91	111	0.8	125	181	79	53	36	88	0.53	4.5
31	35	1	110	72	164	76	28.9	89	98	0.9	215	178	126	43	35	88	0.61	9.3
32	27	1	100	64	164	96	35.6	105	107	0.98	251	310	160	36	62	70	0.93	24.6
33	33	1	110	72	176	91	29.2	107	108	0.99	227	289	159	35	57	85	0.91	24.3
34	34	1	110	64	158	70	28.2	89	102	0.89	214	182	109	46	36	83	0.59	8.5
35	35	1	120	80	161	89	34.1	94	95	0.99	170	213	145	34	42	98	0.79	15.6
36	30	2	126	82	151	67	29	97	111	0.86	184	181	93	47	36	83	0.58	7.7
37	31	2	126	80	158	75	30.5	99	112	0.88	284	210	132	45	42	82	0.66	9.4
38	35	2	110	70	158	74	29.8	97	117	0.8	284	190	100	47	38	82	0.6	7.9
39	35	1	126	78	163	80	30.2	98	106	0.92	198	205	128	41	41	84	0.69	9.2
40	34	1	126	78	165	88	32.2	96	97	0.99	150	285	144	36	57	78	0.89	13.3

MASTER CHART - CASES

S.NO	AGE	SEX	Sys .BPmmHg	Dias.BP mmHg	Height cm	Weight kg	BMI	Waist circumference cm	Hip circumference cm	Waist / Hip Ratio	T.CHO mg/dl	TRIGLY mg/dl	LDL mg/dl	HDL mg/dl	VLDL mg/dl	FBS mg/dl	Atherogenic Index	Plasma Vitatin ng/ml
41	31	1	118	70	168	81	28.4	88	118	0.8	226	213	98	48	42	81	0.64	7.9
42	31	1	120	80	172	82	27.9	89	114	0.85	172	219	118	44	43	93	0.69	8.7
43	35	1	118	76	156	79	32.5	92	103	0.95	218	226	144	36	45	92	0.79	11
44	19	2	108	76	152	66	28.5	96	110	0.86	203	187	119	45	37	70	0.61	8.3
45	30	2	126	78	159	73	28.9	97	104	0.92	177	231	128	40	46	98	0.76	9.2
46	22	2	120	70	154	64	26.9	91	111	0.8	212	181	79	53	36	88	0.53	4.5
47	33	2	116	72	159	73	28.9	92	100	0.82	218	164	99	48	33	90	0.53	7.1
48	33	2	116	72	155	70	29.1	94	109	0.85	213	210	108	43	42	99	0.68	8.2
49	35	2	100	80	164	90	33.9	100	107	0.93	432	258	151	35	51	80	0.86	23.9
50	33	1	116	70	162	75	28.6	95	113	0.89	182	176	117	42	35	86	0.62	9.2
51	35	2	110	70	158	92	36.2	118	125	0.93	227	240	146	33	48	90	0.86	33.2
52	35	2	120	70	159	76	30.2	96	109	0.88	180	175	120	41	35	82	0.63	9.6
53	30	2	114	68	160	110	43.7	98	104	0.94	190	234	261	28	46	88	0.92	69.8
54	31	1	118	70	165	80	29.8	95	108	0.87	182	191	132	42	38	86	0.65	9.7
55	32	2	120	70	156	80	32.4	102	110	0.92	195	176	182	35	35	91	0.7	20
56	33	2	100	78	160	102	39.5	116	118	0.98	181	210	224	33	42	86	0.8	52.2
57	35	2	100	72	154	72	30.8	96	105	0.91	399	144	138	40	28	84	0.55	10
58	30	1	98	60	161	74	28.9	87	101	0.86	188	240	119	46	48	95	0.71	8.7
59	29	2	100	68	162	86	32	92	100	0.92	185	257	149	36	51	88	0.85	14.6
60	30	2	96	70	158	69	27.9	91	105	0.82	188	165	87	56	33	87	0.27	6.4

MASTER CHART -CONTROLS

S.NO	AGE	SEX	Sys .BPmmHg	Dias.BP mmHg	Height cm	Weight kg	BMI	Waist circumference cm	Hip circumference cm	Waist / Hip Ratio	T.CHO mg/dl	TRIGLY mg/dl	LDL mg/dl	HDL mg/dl	VLDL mg/dl	FBS mg/dl	Atherogenic Index	Plasma Visfatin ng/ml
1	26	1	126	82	160	52	20.2	71	87	0.81	138	84	79	40	41	90	0.32	8.5
2	34	1	130	72	168	68	24.4	68	76	0.89	144	63	75	41	12	99	0.18	5.7
3	30	1	110	70	170	60	20.5	72	84	0.85	135	102	78	31	20	80	0.21	8.8
4	28	1	100	68	172	60	20	75	80	0.93	118	58	62	39	11	86	0.17	7.7
5	30	1	110	80	168	65	23.2	78	85	0.91	112	105	70	21	27	90	0.69	3.4
6	34	1	118	80	168	58	20.8	72	84	0.85	167	54	88	52	11	82	0.01	6.3
7	25	1	116	78	166	59	20.9	71	83	0.85	124	49	66	41	10	88	0.07	7.5
8	25	2	110	80	152	56	24.8	70	89	0.78	150	81	85	52	17	83	0.19	5.3
9	34	1	120	78	166	62	22.6	73	90	0.81	150	108	95	43	21	91	0.39	6.7
10	35	2	110	80	161	60	23.5	74	89	0.83	100	108	104	43	28	79	0.43	8
11	29	1	118	70	166	61	21.4	75	87	0.86	153	102	84	39	20	84	0.41	6
12	20	1	128	78	168	62	22.9	74	90	0.82	156	91	81	44	18	95	0.31	5.9
13	35	2	110	70	156	54	23.8	73	87	0.83	155	94	84	35	19	88	0.42	6.3
14	28	1	120	80	174	56	18.3	72	86	0.83	136	105	65	45	21	86	0.35	4.6
15	34	2	120	76	158	60	24.1	71	87	0.81	108	81	97	45	16	97	0.25	7.2
16	35	1	120	80	160	63	24.4	78	89	0.87	104	89	82	49	18	80	0.2	5.3
17	34	1	112	76	164	60	22.6	76	86	0.88	160	96	88	47	18	85	0.42	6.7
18	31	1	110	70	164	60	22.6	75	90	0.83	165	109	97	47	22	87	0.34	8
19	32	2	120	78	164	64	23.5	82	96	0.85	155	70	96	38	14	85	0.26	5.9
20	35	2	120	80	170	63	22.9	79	89	0.88	148	81	90	34	16	83	0.37	7.1

MASTER CHART -CONTROLS

S.NO	AGE	SEX	Sys .BPmmHg	Dias.BP mmHg	Height cm	Weight kg	BMI	Waist circumference cm	Hip circumference cm	Waist / Hip Ratio	T.CHO mg/dl	TRIGLY mg/dl	LDL mg/dl	HDL mg/dl	VLDL mg/dl	FBS mg/dl	Atherogenic Index	Plasma Visfatin ng/ml
21	28	1	120	76	172	60	20.5	76	87	0.87	151	89	97	45	18	82	0.29	5.7
22	34	1	80	60	165	60	22.8	72	86	0.83	140	81	82	33	16	72	0.38	5.6
23	32	1	120	78	162	61	23.5	79	90	0.87	146	81	82	33	16	84	0.38	4.8
24	35	1	100	70	165	64	23	82	90	0.91	140	81	82	33	16	78	0.38	8.5
25	34	2	110	76	158	56	22.6	71	87	0.81	137	66	83	39	13	81	0.22	5.7
26	34	2	100	60	156	50	20.5	70	87	0.8	105	58	55	38	11	93	0.18	8.8
27	35	2	100	70	154	51	21.2	73	89	0.82	145	91	99	42	18	92	0.33	4.6
28	32	2	90	60	158	55	22.9	80	91	0.87	156	85	102	42	17	70	0.3	7
29	35	2	116	70	165	66	24.1	80	92	0.86	123	97	94	43	21	98	0.35	5.2
30	29	2	90	70	158	46	18.3	70	93	0.75	103	59	52	32	11	84	0.26	8.8

Chennai Medical College Hospital & Research Centre

Irungalur, Trichy – 621 105.

Consent form

You are requested to participate in a study conducted in the department of biochemistry, Chennai medical college hospital & research centre, Irungalur, Trichy, Tamilnadu titled "**A Study of Serum Visfatin Levels, Atherogenic Index and Body Fat Distribution in Young Obese Adults"**. your participation in the study is voluntary.

- There will be no cost for participating in the study
- Your participation is not a compulsion
- You have the right to withdraw from the study at any time.

Nature of study:

- ✓ If any abnormalities are identified, you will be informed for further consultation.
- ✓ The results of this study will be kept confidential

We believe that the results of this study will be beneficial for advancements in medicine & science. We assure you that we will not use these result for any other purpose.

Consent

I mr /mrs / ms_____residing at _____on this day _____ after having read the consent form carrying information for the above mentioned study and i hereby give my consent to take 5ml of my blood sample for the purpose of doing serum visfatin levels, and lipid profile to calculate my atherogenic index. I also give consent to measure my height, weight waist and hip circumference for this study purpose. I was explained about the procedure in detail and give my consent for participating in the study and for using the results for medical & scientific purposes.

Signature of the participant signature of the investigator

சென்னை மருத்துவக்கல்லூரி மருத்துவமனை மற்றும் ஆராய்ச்சி மையம்,

இருங்களூர், திருச்சிராப்பள்ளி – 621 105

ஒப்புதல் படிவம்

சென்னை மருத்துவக்கல்லூரி மருத்துவமனை மற்றும் ஆராய்ச்சி மையத்தின் உயிர் வேதியியல் துறையில் நடத்தப்படும். "உடல் பருமன் உள்ளவர்களின் விஸ்பாட்டின் அளவையும் கொழுப்பின் அளவையும் கண்டறிதல் – ஓர் ஆய்வு"பங்கேற்குமாறு உங்களை கேட்டுக் கொள்கிறோம்.

- இப்பரிசோதனைக்கு சம்மதிப்பது உங்கள் விருப்பத்தைப் பொறுத்தது.
- இச்சோதனைக்கு கட்டணம் கிடையாது.
- கட்டாயம் ஏதும் இல்லை.
- பரிசோதனையிலிருந்து எந்நேரமும் விலக தங்களுக்கு முழு உரிமை உண்டு.

இந்த ஆய்வின் முடிவுகள் மருத்துவம் மற்றும் விஞ்ஞான முன்னேற்றத்திற்கு உதவும் என்று கருதுகின்றோம். இவைகளை வேறு எதற்கும் பயன்படுத்தப்பட மாட்டாது என உறுதியளிக்கிறோம்.

ஒப்புதல்

நான்திரு/திருமதி/செல்வி/

_____முகவரி_____

_____

_____நாள்_____

அன்று மேற்கண்ட ஆய்வுக்காக தகவல் படிவத்தினை படித்து, கேட்டு புரிந்து கொண்டு இந்த ஆராய்ச்சிக்கு தேவையான சோதனைக்கு என்னிடம் இருந்து 5மிலி. இரத்தத்தை காலை உணவிற்கு முன்புஎடுத்துக் கொள்ள அனுமதிக்கிறேன்.என மனப்பூர்வமான சம்மதத்தை அளிப்பதோடு இந்த ஆய்வின் முடிவுகளை மருத்துவம் மற்றும் விஞ்ஞான நோக்கத்திற்கு பயன்படுத்த ஒப்புதல் அளிக்கிறேன்.

நடுநிலை சாட்சியின் கையொப்பம்பங்கேற்பாளர் கையொப்பம்

ஆய்வாளர்/சம்மதம் பெறுபவர் கையொப்பம்